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Basle Hypertension Symposium

*Proceedings of a symposium held at Sandoz AG Basle
December 13th and 14th 1976*

Edited by Hans Dunér

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OPENING ADDRESS

This symposium is devoted to hypertension—a field in medicine in which over the last two decades much progress has been made on both the epidemiologic and the therapeutic levels. Due to its high prevalence and its serious socio-economic implications hypertension has been recognised as a major community health problem for which the solution lies to a great extent in early diagnosis and subsequently adequate treatment.

Although, I am speaking for a pharmaceutical firm, which with its past and present research activities, is heavily engaged in the search for and development of new antihypertensive agents, I do by no means pretend that drug therapy is the only possible approach to the problem. A more healthy way of life with less exposure to stress, with reduced food consumption and diminished salt intake would certainly make a very important contribution but I feel quite safe in saying that at the present time and for the next few years—may be even decades—drug therapy will continue to have a better chance of reducing the problem of hypertension than any attempt to alter communities' living habits.

This is even more the case since the use of modern antihypertensive drugs—and I am referring here mainly to the β -adrenoceptor blocking agents—can be based on a very much improved understanding of the underlying pathophysiological and in particular haemodynamic principles of hypertension, and cause much less discomfort to patients than the older drugs.

Since I was a pharmacologist in my earlier years, I am still intrigued by the question of whether and to what extent results obtained in animals can be applied to man. There is growing evidence that in man early and proper antihypertensive therapy reduces the risk of stroke, cardiac and renal insufficiency and possibly also of myocardial infarction, thereby prolonging the period of enjoyable life and lowering mortality of the disease. In this context I would like to show very briefly two figures which illustrate results obtained by Dr Salzmann from our Pharmacology Department on the effects of early treatment of experimentally induced hypertension in rats.

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Effect of pindolol (Vakern) on stroke blood pressure in the Grollman rat

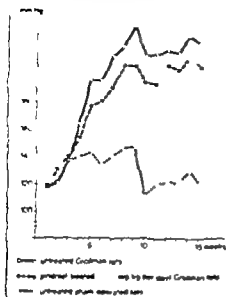


Fig 1

Effect of pindolol (Vakern) on survival of Grollman rats

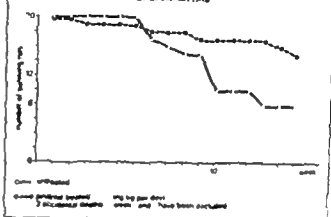


Fig 2

Figure 1 shows the development of blood pressure in untreated and pindolol treated Grollman rats and in sham-operated untreated animals. The drug was given in a daily dose of 0.5 mg/kg in the food from the time when the first surgical procedure on one kidney was performed until the end of the 15th week after surgery. Compared with the results from other animal models for instance the spontaneously hypertensive rat or DOCA and salt induced hypertension the reduction in blood pressure achieved by pindolol in the Grollman rat was not very impressive and in most measurements not statistically different from non treated controls.

However as shown in Figure 2 the small differences may have been of some therapeutic significance. Here the number of surviving animals is plotted against time. Clearly β receptor blockade resulted in an improved survival rate and it is of course tempting to speculate that this protection was at least partially due to the modest reduction in arterial blood pressure.

It would be of interest to go into more details of this and similar studies and to discuss the predictability of animal experiments for the human use of antihypertensive drugs. However this is not the topic of today's discussion and I would therefore like to conclude by thanking all of you for having accepted the invitation to come to Basle and by expressing our sincere gratitude to the four chairmen and all the speakers for their willingness to actively participate in our symposium.

The Care of the Hypertensive Patient

Chairman Gösta Tibblin

Department of Social Medicine University of Umeå Umeå, Sweden

Effect of pindolol (Vascore) on systolic blood pressure in the Grollman rat

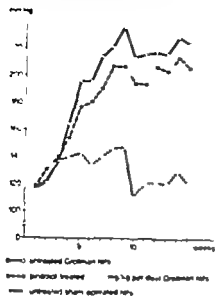


Fig 1

Effect of pindolol (Vascore) on survival of Grollman rats

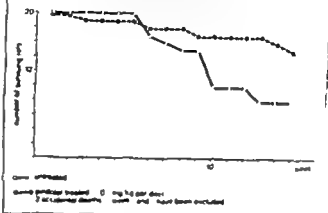


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Treatment of Hypertension in the Community

A preliminary report

Göran Berglund, Ove Andersson and Lars Wilhelmsen

*From the Department of Medicine I Sahlgrenska Hospital University of Göteborg
Göteborg Sweden*

Abstract. The feasibility and efficacy of antihypertensive treatment within the Primary Prevention Trial against myocardial infarction and stroke in Göteborg, Sweden, are described. The study group consisted of 9 967 men aged 47–54 years, chosen at random from the general population. 75% of them participated in a screening examination including a blood pressure (BP) measurement. Hypertension defined as BP above 175 mm Hg systolic or 115 mm Hg diastolic on two separate occasions or antihypertensive treatment was found in 9%.

The drop-out rate was 6.5% during the first year and 3.6% during the second year of treatment. The percentage of hypertensive subjects with uncontrolled BP decreased from 83% at screening to 16% after one year and to 9% after 10 years of treatment. The frequency of side-effects of such severity as to cause withdrawal of drugs, was 16% during the first year and 7% during the second year.

The morbidity in coronary heart disease and stroke in those hypertensives ($n=696$) treated at the Hypertension Clinic was compared with that of an untreated control group ($n=338$). The incidence of death and fatal plus non-fatal myocardial infarction was significantly lower in the treated group than in the untreated comparison group in spite of lower initially predicted risk of myocardial infarction in the comparison group. The findings indicate that antihypertensive treatment prevent the occurrence of coronary heart disease.

In selected groups of hypertensive subjects (15–16) antihypertensive treatment has been shown to prevent the development of stroke and other diseases directly related to increased blood pressure (BP). These studies were unable, however, to show

an effect of treatment on the incidence of coronary heart disease. Furthermore the feasibility of keeping large populations under treatment for long periods of time with low drop-out and side effect rates and with acceptable BP control has been questioned (1).

This paper presents the feasibility and efficacy of antihypertensive treatment at a specialized Hypertension Clinic in an unselected male middle aged Swedish population.

STUDY POPULATION AND METHODS

The present study population was recruited from a screening examination which was part of a primary preventive trial started in 1970 (18). Pertinent data on the study population and trial design are given in Figure 1. Details of the screening examination as well as the BP criteria for further action, such as diagnostic investigation and treatment, have been described previously (19). Briefly

The Primary Preventive Trial, Göteborg, Sweden

Main objective: Intervention

End-point for evaluation: Mortality: total and cause specific
Morbidity: from CHD and stroke

Duration of the trial: c. 1970–1977

Study group: General population: 10 000 M, 47–54 years

Control group: General population: 20 000 M, 47–54 years

Definition of HT: >175 and/or 115/2 mmHg on two occasions or treatment

Clinic finding made by non-continuous, specialized clinic

Treatment: Study group: No standardized protocol

Control group: No clinic finding done

Treatment provided in specialized clinic

Educational measures directed towards 11 preventive subjects

Fig. 1 Data on study population and methods.

CHD=Coronary heart disease. HT=Hypertension.

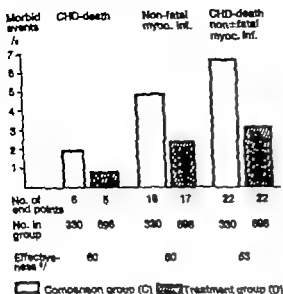


Fig 4 The percentage incidence of death from coronary heart disease and non-fatal myocardial infarction in the Comparison group (C) and the Treatment group (T)

Table 1 The drop-out rate during the first and second year of treatment at the Hypertension Clinic. The Primary Preventive Trial, Göteborg, Sweden ($n=696$)

	1st year	2nd year
	% n of 696	% n of 696
Remaining under control (the Hypertension Clinic)	646 94.2	624 91.0
Drop-outs	40 5.8	22 3.2
Refused	5	2
Deceased	17	8
Moved	7	4
Died	6	5
Other reasons	3	3

Table 2 The frequency of good acceptable and poor blood pressure control after one and two years of treatment at the Hypertension Clinic. The Primary Preventive Trial, Göteborg, Sweden ($n=696$)

	1st year	2nd year
	% n	% n
Treated	610	589
BP-control		
<160 and 95 mm Hg	399 33	262 44
160-175 or 95-115 mm Hg	31 51	78 47
>175 or 115 mm Hg	99 16	51 9

The drop-out rate

During the first year 5.8% and during the second year 3.2% dropped out of treatment (Table 1). These figures include drop-outs due to death and migration (2.0% during the first year and 1.3% during the second year).

The BP control

At the screening examination 83% of the hypertensive subjects had BP above the limits for hypertension (Figure 3). The corresponding figures after one and two years of treatment were 16% and 9% (Table 2). Only 33% and 44% respectively had definitely normal BP i.e. below 160 mm Hg systolic and 95 mm Hg diastolic.

Side-effects causing withdrawal

During the first year 16% and during the second year 7% experienced side-effects of such severity as to cause withdrawal of the drug and institution of alternative therapy (Table 3). In the great majority of cases the reported side-effects could not be attributed to drug-specific effects.

Effects of the treatment on cardiovascular events

Twenty-two of the 696 hypertensive men (3.2%) treated and followed up at the Hypertension Clinic had a fatal or a non-fatal myocardial infarction during the follow-up period (Figure 4). The corresponding figure in the untreated comparison group was 22/330 (6.7%). The difference was statistically significant ($p<0.05$). When fatal plus non-fatal strokes were added ("all cardiovascular events") there was still a statistically significant difference ($p<0.05$).

There was a significant difference in total mortality ($p<0.05$) while no significant difference was found for mortality from coronary heart disease or stroke between the hypertensive subjects treated at the Hypertension Clinic and the untreated control group.

Table 3 The frequency of side-effects of such severity as to cause withdrawal of the drug. Results are given separately for the first and second year of treatment.

	1st year	2nd year
	n %	n %
On treatment	610	589
Withdrawal due to side-effects	98 16	38 7

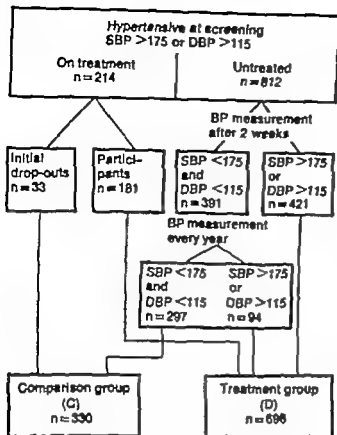


Fig Creation of a Comparison group (C) and a Treatment group (D)

Definition of hypertension

BP >175 or 115 twice
or antihypertensive treatment
(n=686)

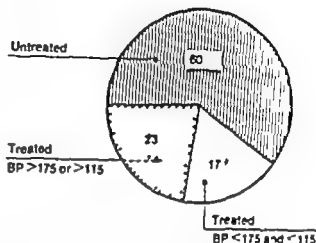


Fig 3 The prevalence of hypertension and the proportion of treated and untreated hypertensive subjects

blocker in the great majority of patients adding a saluretic diuretic or hydralazine or both if normotension has not been achieved. The clinic was responsible for giving appointments to the patients and those who failed to attend an appointment were actively sought out in order to return them for further follow-up.

Non fatal and fatal myocardial infarctions and strokes, and all deaths including sudden deaths, were followed by means of registers covering all cases in Göteborg (7-9). The morbidity analysis covers a follow-up period from the start of the study in 1970 until December 31st 1975.

Statistical methods used included Chi squared test for comparison of proportions and Student's *t*-test for comparison of mean values. A multiple risk factor was assigned to the subjects with the aid of a computer according to a multiple logit function described previously (10).

RESULTS

Prevalence of hypertension

Hypertension defined as BP above 175 mm Hg systolic or 115 mm Hg diastolic on two separate occasions or current antihypertensive treatment was found in 686 men (9%). Of these 14% were untreated and 86% were treated but had BP above the cut off points for hypertension. Only 17% had an acceptable BP control, i.e. BP below 175 mm Hg systolic and 115 mm Hg diastolic (Figure 3).

of the 9 967 47-54 year-old men invited to attend 75% took part in a screening examination. Those with BP above 175 mm Hg systolic or 115 mm Hg diastolic on two separate occasions and those on antihypertensive treatment (n=686) were subjected to a standardized diagnostic investigation (19). Those with BP above the limits for hypertension at screening but not at the BP check up two weeks later were recalled for another BP check-up after one year. The criteria for referral for treatment are given in Figure 2. This figure also shows how a treated and an untreated group were created for the comparison of mortality and morbidity. It is worth noting the difference in composition between the group of 696 men according to Figure 2 and the group of 686 men described in Figure 3. The treatment group in Figure 2 includes the subgroup of 94 men who were found to be hypertensive at yearly BP-checkups, while the latter is a group of well treated hypertensives (SBP <175 and DBP <115).

After the diagnostic investigation at the Hypertension Clinic the hypertensive subjects were put on hypotensive treatment. Although the treatment policy has not been rigorously uniform from 1970 until 1976 treatment has been started with a beta

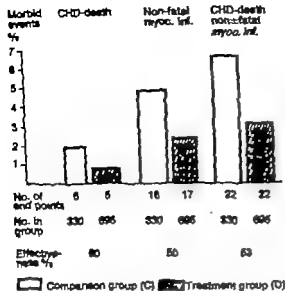


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Twenty-two of the 696 hypertensive men (3.2%) treated and followed up at the Hypertension Clinic had a fatal or a non-fatal myocardial infarction during the follow-up period (Figure 4). The corresponding figure in the untreated comparison group was 22/330 (6.7%). The difference was statistically significant ($p<0.05$). When fatal plus non-fatal strokes were added ("all cardiovascular events") there was still a statistically significant difference ($p<0.05$).

There was a significant difference in total mortality ($p<0.05$) while no significant difference was found for mortality from coronary heart disease or stroke between the hypertensive subjects treated at the Hypertension Clinic and the untreated control group.

Table 3 The frequency of side-effects of such severity as to cause withdrawal of the drug. Results are given separately for the first and second year of treatment.

	1st year	2nd year
	%	%
The Primary Preventive Trial, Göteborg, Sweden ($n=686$)		
On treatment	610	589
Withdrawal due to side-effects	16	7

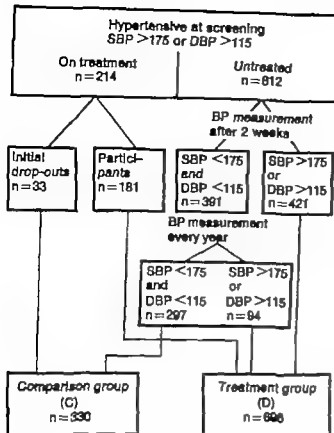


Fig 2 Creation of a Comparison group (C) and a Treatment group (D)

of the 9 967 47–54 year-old men invited to attend 75% took part in a screening examination. Those with BP above 175 mm Hg systolic or 115 mm Hg diastolic on two separate occasions and those on antihypertensive treatment ($n=686$) were subjected to a standardized diagnostic investigation (19). Those with BP above the limits for hypertension at screening but not at the BP check up two weeks later were recalled for another BP check-up after one year. The criteria for referral for treatment are given in Figure 2. This figure also shows how a treated and an untreated group were created for the comparison of mortality and morbidity. It is worth noting the difference in composition between the group of 696 men according to Figure 2 and the group of 686 men described in Figure 3. The treatment group in Figure 2 includes the subgroup of 94 men who were found to be hypertensive at yearly BP-checkups, while the latter is a group of well-treated hypertensives (SBP <175 and DBP <115).

After the diagnostic investigation at the Hypertension Clinic the hypertensive subjects were put on hypotensive treatment. Although the treatment policy has not been rigorously uniform from 1970 until 1976, treatment has been started with a beta

Definition of hypertension

BP >175 or 115 twice
or antihypertensive treatment
($n=686$)

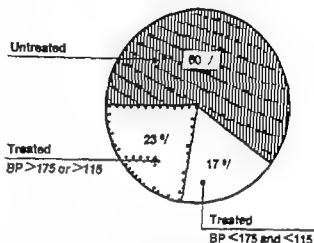


Fig 3 The prevalence of hypertension and the proportion of treated and untreated hypertensive subjects.

blocker in the great majority of patients, adding a saluretic diuretic or hydralazine or both if normotension has not been achieved. The clinic was responsible for giving appointments to the patients and those who failed to attend an appointment were actively sought out in order to return them for further follow-up.

Non-fatal and fatal myocardial infarctions and strokes, and all deaths including sudden deaths, were followed by means of registers covering all cases in Göteborg (7–9). The morbidity analysis covers a follow-up period from the start of the study in 1970 until December 31st, 1975.

Statistical methods used included Chi-squared test for comparison of proportions and Student's *t*-test for comparison of mean values. A multiple risk factor was assigned to the subjects with the aid of a computer according to a multiple logistic function described previously (20).

RESULTS

Prevalence of hypertension

Hypertension defined as BP above 175 mm Hg systolic or 115 mm Hg diastolic on two separate occasions or current antihypertensive treatment was found in 686 men (9%). Of these 60% were untreated and 3% were treated but had BP above the cut off-points for hypertension. Only 17% had an acceptable BP control i.e. BP below 175 mm Hg systolic and 115 mm Hg diastolic (Figure 3).

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The community has about 7000 inhabitants, of which 5370 are over 25 years of age. It has 2 out-patient doctors in the village Edsbyn and that is why the study is called "The Edsbyn study".

The aim of the study was to get information on the amount of medical care resources necessary to take care of all the hypertensives, partly by making a screening examination and partly by using a scheduled programme for hypertension at the outpatient clinic.

We would now like to present some of the results of the study which have aroused our special interest. The screening programme comprised a short interview, blood pressure, arm circumference and pulse rate. The blood pressure observer put questions in connection with the blood pressure measurement. The interview comprised questions about heredity for vascular disease or diabetes, earlier known hypertension or diabetes, therapy for blood pressure, time for last visit to the outpatient clinic and habits in regard to tobacco, alcohol and exercise.

The blood pressure observation was made with the patients in a recumbent position without rest during daytime. One single observer made the screening and she had been tested with several other observers as to the reliability of the readings and did not differ from the rest of the group. She had a detailed description and used a mercury manometer. The balloon in the cuff was 12×35 cm. The readings were made per 2 mm and the diastolic pressure read in phase 5.

The population studied was completely unselected and comprised everyone over 25 years of age, that is 2626 men and 2745 women. 87 per cent of the men and 85 per cent of the women were examined. In certain age groups the participation rate was more than 90 per cent.

The criteria for selection were unknown to the nurse who made the screening. The limits had been set very low in order to give the opportunity to make a thorough evaluation of hypertensives in the area.

The result of the screening was unexpectedly low: 4.4 per cent of the men and 2.8 per cent of the women. In total 166 persons had abnormal screening findings. Among these there were 53 known hypertensives. Of the remaining 113 only 34 fulfilled the criteria for therapy, i.e. in the scheduled programme were put at more than 105 mm mercury diastolic and, for patients over 60 years of age, more than 110 mm. (Mean of three separate readings, the screening excluded). After carefully going through all the papers at the out-

No difference in age cholesterol or smoking habits at entry was found between the treated group and the untreated comparison group

DISCUSSION

We have chosen to study men within a narrow age span in order to minimize any influences of age and sex on the variables studied. The study population has been drawn so that the results can be applied to other male populations with a similar background. This is acceptable if the non participation group does not differ substantially from the population studied. Earlier epidemiological studies by our group have shown that the non participation group may differ in respect of socioeconomic factors (13) and mortality (20). The non-participation rate in the present study was 25%. Similar or even higher rates have been reported in certain other population studies (3, 5, 6, 8). Other studies, however, have shown higher participation rates (11, 13, 14). The present study, which is a primary preventive trial, was designed so that it would be possible to repeat it on a larger scale if the results proved favourable. Further efforts have therefore not been made to get hold of those who did not answer two letters of invitation. It has been supposed that those who were unwilling to attend a screening examination would also be reluctant to comply with treatment regimens.

The limits for hypertension (175/115 mm Hg in the afternoon) may appear to be high. Results of BP measurements in the morning (167/101) (19) the distribution of patients by WHO stage and findings concerning cardiac (17) and renal function (2) all indicate, however, that most hypertensives studied had mild to moderate hypertension without serious heart or kidney damage at entry.

The descriptive BP data are in good agreement with earlier studies in Göteborg (19) and elsewhere (10) showing that a majority of persons with hypertension were untreated and that a large proportion of those on treatment had high BP.

The programme for control of hypertension proved to be effective in lowering BP in the majority of hypertensives. However, there still remained a substantial proportion of patients whose BP could not be adequately controlled despite heavy antihypertensive drug therapy. Obviously, there is a great need for further knowledge of the

cause of the poor BP response in this group. More potent antihypertensive drugs are also needed. The BP decrease was achieved with an acceptable frequency of side-effects, the drug withdrawal rate due to side-effects being fully comparable with that in another large-scale hypertension study (17). The drop-out rate was extremely low compared to conventional management (1, 4).

The results regarding the incidence of cardiovascular events indicate a preventive effect of the programme on the occurrence of coronary heart disease. One must remember, however, that this is not a strict double blind trial with a treatment group and placebo-treated control group. It is therefore not possible to ascribe the preventive effect solely to the drug regimen used as beneficial effects from the closer supervision of the treated group cannot be ruled out. Further evaluations will disclose whether this was the case. The difference found cannot be explained by different risk patterns in the two groups as no differences were found in the usual risk factors for coronary heart disease.

This study shows that BP treatment, in the hands of specialists, is feasible and that it also seems to be effective in reducing the morbidity from cardiovascular diseases.

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patient clinic, 480 hypertensives were found who had received therapy before the screening.

However the criteria for the diagnoses hypertension and for therapy among these were probably not exactly the same as the above mentioned but they are considered hypertensives in this study.

That gives a figure of 10 per cent hypertensives in the population over 25 years of age.

The diastolic pressures for men age 50-54 have been compared with some other wellknown studies but there are at present no comments as to the differences.

Special interest has been paid to the non participating group. As expected the youngest and the oldest are in majority here. Of 764 or 14 per cent of the studied population that did not participate in screening it has been possible to get information on blood pressure for 533. That means that it has been possible to estimate the prevalence of hypertension for 96 per cent of the studied population.

The following conclusions have been drawn

1. A well run outpatient care organization in Sweden in this case one doctor per 3500 inhabitants, is able to detect, take care of and treat all the hypertensives in the population from the quantitative point of view.

2. With a strictly scheduled programme with fixed criterias for therapy, therapy goals, scheduled routines for examination and check ups, and with the possibility of delegating certain tasks to a nurse it is possible to cover patients with other diagnosis as well.

3. A screening procedure gives very poor results where outpatient care is well established and is probably only of value initially.

Bengt Schersten

I have a question to Göran Berglund. How was the blood pressure measured? During screening II was measured in the sitting position. I think. How was it measured during the control?

Göran Berglund

During the controls it was measured after five minutes rest in the sitting position. At the screening the rest was 3-5 minutes, during which time an interview was carried out. Thus they are quite comparable.

John-Fredrik Dymling

Did you make any attempt to compare the morbidity of the groups, which did not have well controlled hypertension? There were quite a few people in these groups.

Göran Berglund

The end point numbers are still fairly small. In the treated group and in the comparison group only about 20 up till now had a myocardial infarction. Further subdivision into those who had

normalised blood pressure and those who still had high blood pressure would make a statistical analysis impossible.

Johan Asplund

How many of your patients in the treated group were on beta blockers?

Göran Berglund

Beta-blockers are the first choice drugs at our clinic. About 75% of the patients are on a beta-blocker now.

Lennart Sölvell

The frequency of side-effects was about 25% in two years. Could you tell us anything about the type of side-effects?

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We had about the same frequency of side-effects in the saluretic treated patients and in the beta blocker treated patients. The usual cause of withdrawal in the saluretic group was hypokalemia, in spite of a heavy potassium supplement. In some patients gout also occurred. In the beta-blocker group there were more unspecific types of side-effects. It was very hard to say if they were drug related or not. In one or two cases we found a heart decompensation.

Gösta Tibblin

As you said this is neither a double blind study and nor a controlled randomised study. What kind of differences could exist between the two groups, the control group and the experimental group? Perhaps the control group has a more variable blood pressure? We know from our own study that a large variability in the pressure can be a risk factor in itself. The other question is whether there are any differences in smoking habits, in cholesterol and so on between the two groups?

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We have not yet finished the rescreening of the whole group. That will be done in 1977. However it is quite clear that there is a more variable blood pressure in the control group. The treatment group must have had a more stable blood pressure since the criterion was two blood pressure measurements of 175/115 or more. It is possible that a variable blood pressure is a more pronounced risk factor than a stable blood pressure. We cannot answer that question.

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It has been stressed repeatedly that our medical care resources are not large enough to allow proper treatment of hypertension in the population. The results of the Edhyn study imply that the situation may not be bad and that a large

proportion of the Swedish population may already have proper antihypertensive care.

Sven-Olof Jacobson

I can agree with Sören Jakobsson to some extent, but overall our experience in Skaraborg county is that we have about the same proportion of treated cases as you have in Gothenburg. In most communities we have around 15% on adequate treatment and the rest are not treated or on inadequate treatment.

Göran Bergholm

Did you have a physician in Edsbyn, who was particularly conscientious and who had been established for many years, which could explain the low frequency of unknown hypertensives?

Lars Sundman

Maybe this was a factor but it is by no means clear. The physician had been working in Edsbyn for only two years when we started. We are going to carry out a similar study in another part of our county to see what situation we have there.

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I think it is very important to realize that Edsbyn is certainly not representative for primary medical care in the whole of Sweden. In the north, we are still short of doctors, and can certainly not deliver such good primary medical care.

Berni Hokfelt

Would you care to comment on the prevalence of hypertension in a city as compared to a rural district?

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I cannot answer your question specifically but it is our impression, that blood pressure levels in the Edsbyn region are lower than those in the city of Gävle. However I have no comments at all as to why this is so.

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Long-term Aspects on Essential Hypertension

Ingvar Liljefors

From the Department of Internal Medicine, St Görans Hospital,
Stockholm, Sweden

Abstract. Hypertension has been found to imply a disease entity since the beginning of this century. Effective treatment available in the last two decades has changed the outlook remarkably especially in malignant hypertension.

There are more and more evidence that less severe forms of hypertension will benefit from keeping the blood pressure under control.

In 1913 Dr Theodore C. Janeway (8) read a paper before the Association of American Physicians on a series of nearly 500 patients with hypertension seen in his private practice and followed for 9 years. Based on this study he then made a statement which is still valuable.

It does not seem to me that any very definite prognostic conclusions can be drawn from the height of the blood pressure. This paradox has been evident to all physicians up to the present time when a high blood pressure is revealed.

The existence of some patients with well defined diseases, such as nephritis, aortic valve insufficiency or thyrotoxicosis was well known. The majority of patients with hypertension did not, however present such etiology and thus the prognostic judgment had to be based on experience of different types of essential hypertension.

Based on the clinical studies of Volhard and Fahr (11), the three representatives of different branches of medicine Keith, Wagener & Barker (9) suggested that the prognosis of hypertensive patients would be recognized by classification of the eye ground pattern. Their study comprised a series of 219 patients followed for 7 years, and it displayed a quite different outlook for the 4 groups (Figure 1). Malignant hypertension with its urgent need for effective treatment, is probably still most clearly identified by recognizing the papilloedema

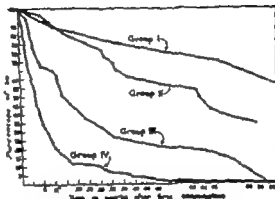


Fig 1 Survival curves in the four groups according to Keith, Wagener & Barker (1939).

of the group IV patients. At the same time when treatment of hypertension was restricted to combatting its effects on various organs, the outlook for the patients with the malignant type was very bad. The average time of survival was less than one year, the main death cause being uraemia. This form of hypertension represents only a small part of the hypertensive population but is still a challenge in the therapeutic efforts. The prognostic value of Keith & Wagener's classification groups I—III has been disputed. The difficulty even for ophthalmic specialists, of classifying the eye ground pattern is well known. We also know that a benign hypertension may suddenly take a malignant course.

The prognosis of hypertensive disease as recorded in clinical series before the new era of hypertensive treatment, varied considerably depending on the criteria for inclusion of patients. Certain characteristics of importance for the future development of the disease have however been recognized. Here again I would like to quote Dr Janeway from 1913: "I have always had the impression that the expectancy of life of in women

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It does not seem to me that any very definite prognostic conclusions can be drawn from the height of the blood pressure. This paradox has been evident to all physicians up to the present time when a high blood pressure is revealed.

The existence of some patients with well defined diseases, such as nephritis, aortic valve insufficiency or thyrotoxicosis was well known. The majority of patients with hypertension did not, however present such aetiology and thus the prognostic judgement had to be based on experience of different types of essential hypertension.

Based on the clinical studies of Volhard and Fahr (11), the three representatives of different branches of medicine Keith Wagner & Barker (9) suggested that the prognosis of hypertensive patients would be recognized by classification of the eye ground pattern. Their study comprised series of 111 patients followed for 7 years, and it displayed a quite different outlook for the 4 groups (Figure 1). Malignant hypertension, with its urgent need for effective treatment, is probably still most clearly identified by recognizing the papilloedema

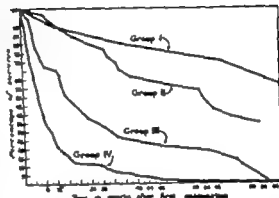


Fig. 1 Survival curves in the four groups according to Keith, Wagner & Barker (1939).

of the group IV patients. At the same time when treatment of hypertension was restricted to combating its effects on various organs, the outlook for the patients with the malignant type was very bad. The average time of survival was less than one year the main death cause being uraemia. This form of hypertension represents only a small part of the hypertensive population but is still a challenge to the therapeutic efforts. The prognostic value of Keith & Wagners classification groups I-III has been disputed. The difficulty even for ophthalmic specialists, of classifying the eye ground pattern is well known. We also know that a benign hypertension may suddenly take a malignant course.

The prognosis of hypertensive disease, as recorded in clinical series before the new era of hypertensive treatment, varied considerably depending on the criteria for inclusion of patients. Certain characteristics of importance for the future development of the disease have, however been recognized. Here again I would like to quote Dr Janeway from 1913 "I have always had the impression that the expectancy of life of the women

Long term Aspects on Essential Hypertension

Ingvar Liljefors

*From the Department of Internal Medicine St Göran Hospital
Stockholm Sweden*

Abstract. Hypertension has been found to imply a disease entity since the beginning of this century. Effective treatment available in the last two decades has changed the outlook remarkably especially in malignant hypertension. There are more and more evidence that less severe forms of hypertension will benefit from keeping the blood pressure under control.

In 1913 Dr Theodore C. Janeway (8) read a paper before the Association of American Physicians on series of nearly 500 patients with hypertension seen in his private practice and followed for 9 years. Based on this study he then made a statement which is still valuable.

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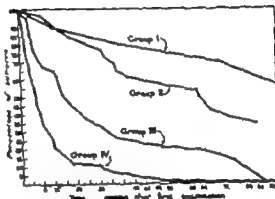


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SYSTOLIC CHANGE

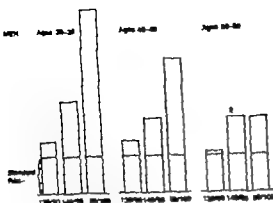


Fig. 3. Blood & Blood Pressure Study (1959).

DIASTOLIC CHANGE

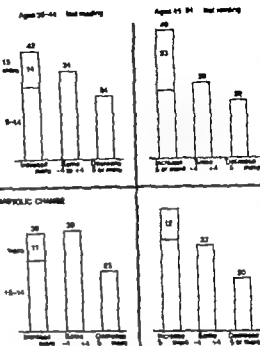


Fig. 4. Long-term changes in blood pressure of individuals; comparison of recent readings of male applicants with their readings taken at least 10-19 years previously (Blood & Blood Pressure Study 1959).

that was revealed in this study namely "the increase with age does not mean a steady rise in blood pressure in every individual, but is the net effect of an increase in some individuals and relatively stationary level in others. As can be seen from Figure 4 less than half of the men and women in the middle age group increase their blood pressure substantially.

Introduction of potent drugs for the control of hypertension has offered a quite different prognosis of most significance to the patients with malignant hypertension. In Sweden we all know Bertil Hood as one of the most devoted advocates of the active treatment of malignant hypertension. Hood and his collaborators, at the beginning of the sixties, were able to present a material of 381 patients of whom 282 were adequately treated (2).

The survival rate of the 72 severe cases of Keith & Wagener grade IV is compared with less active treatment of the same grade of hypertension (Figure 5). There is no doubt that a great number of lives were saved using the new therapeutic possibilities.

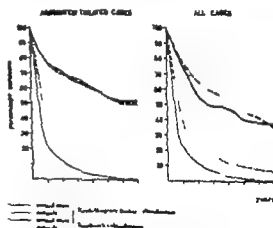


Fig. 5. Survival in treated cases of severe hypertension compared with controls (Björk et al. 1960).

In an attempt to evaluate active treatment of nonmalignant hypertension, Hamilton et al. (7) reported a controlled trial of 61 patients half of whom were given optimal treatment for blood pressure control.

	Age					
	30-39	40-49	50-59	60-69	70+	Total
Males	12.01	4.13	2.54	1.35	0.98	3.01
Females	6.21	5.10	1.91	1.12	0.80	1.62
All persons	7.51	4.91	2.22	1.15	0.87	1.85

Table 1 Observed/expected death rates in hypertension at different ages (corrected for length of follow up) based on 320 deaths in 669 untreated patients (Fry 1974).

FACTORS AFFECTING THE PROGNOSIS IN HYPERTENSION

FAVOURABLY	BADLY
Old parents	Heredity for CHD HT or diabetes
Female sex	Male sex
Age over 60	Age below 50
	Black race
Hypertension known many years	Toxemia Renal disease Cerebrovascular disease
Variable blood pressure	Circulatory dysfunction Weight loss Known risk factors for CHD
Eye grounds I-II	Papilloedema Impaired renal function Cardiac enlargement

Table 2.

with arterial or renal disease was greater than in men. Based on his long experience of over 1 000 cases of hypertension followed for about 40 years, Poul Bechgaard (1) has also pointed out the better outlook for female as apposed to male hypertensives.

An English practitioner Dr John Fry (6), reported in 1974 his experiences with hypertensive patients seen in general practice. Of about 700 patients with a diastolic blood pressure exceeding 100 mm Hg, half of which were less than 120 he followed 669 without treatment for 5 to 20 years. By comparing the death rates of the hypertensives with those of the general population in different age groups, he found the increased mortality to be restricted to the younger age groups especially for males (Table 1).

Apart from age and sex there are certain other factors of importance for the prognosis (Table 2).

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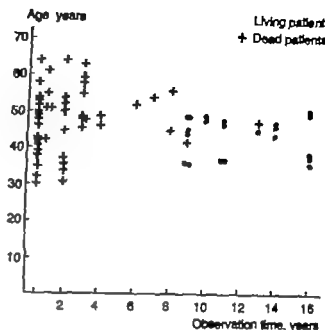


Fig 2 Follow-up of 83 hypertensive patients (Eliassch et al. 1970).

deciding if and how a case of essential hypertension is to be taken care of

Another example of the prognostic variation in nonselected clinical material is Harald Eliassch follow-up study (5) of 83 hypertensive patients investigated around 1950 in St Erik's hospital in Stockholm. The majority of deaths occurred within 3 years of observation (Figure 2). The two main causes of death were cerebrovascular disease and cardiac insufficiency of which the former were dominated by women, and the latter by men. However the cardiac deaths were also associated with patients who were somewhat older at the beginning of the study

Clinical series of hypertensives are for natural reasons, highly selected from the general hypertensive population. Data on people with less severe hypertension is available from epidemiological studies and from insurance companies. One example of the latter is the Build and Blood Pressure Study (4) performed by the Metropolitan Life Insurance Company and based on 4 million people accepted for life insurance. The five times higher mortality compared with normals, for men between 30 and 40 with a quite discrete elevation of blood pressure is striking (Figure 3).

The Build and Blood Pressure Study also shows the wellknown fact that the mean blood pressure level increases with age. In this context, however I think it is well worth pointing out another fact

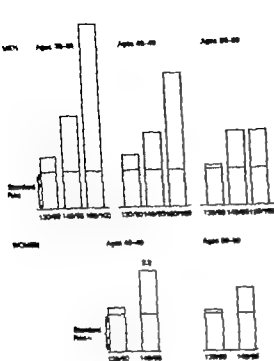


Fig. 3 Bertil & Blood Pressure Study (1959).

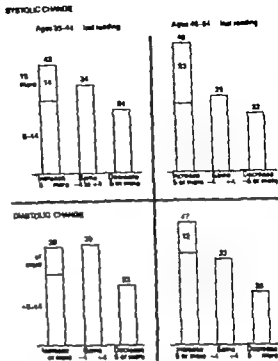


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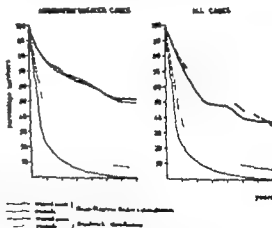


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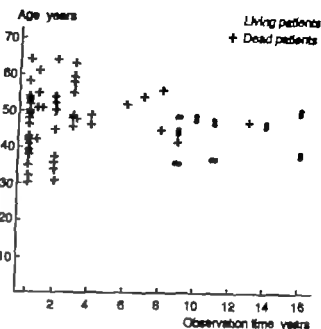


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Table 6. New England Life Mortality Experiences 1954-1972 Borderline and definite hypertension Men, all Ages (Brown & Singer 1976).

Blood Pressure Category Mean	No. of Death Observed	Claims Expected d	Excess Death Rate 1000(d-d')/E	Mortality Ratio 100d/d'
Borderline 141/83	1,539	1,083.8	3.0	142%
Definite Systolic 163/89	112	68.2	5.6	164%
Definite Diastolic 147/100	107	67.5	3.7	199%
Definite S+D 170/103	161	80.4	8.0	200%

sure Study was performed and practically no effective hypotensive treatment was given.

A comparison was made between the two studies which had covered different time periods with different availability of therapy (Figure 6). The differences between the two studies in three of the blood pressure categories are significant at the 5 % level. These trends towards a decrease in the overall mortality may of course have several causes since many other factors have been altered to reduce mortality over the past twenty years. On practical, and by no means uninteresting factor resulting from the availability of new and less risky means of treating hypertension is the chance of keeping blood pressure and subsequently the insurance premium loading, under control.

I will conclude with another quotation from Dr Janeway which is still suitable:

"This illustrates well the advantage of life insurance examination for the early detection of chronic disease.

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Comparison of Mortality Ratios by diastolic blood pressure category Men 40-49 (Brown & Singer 1976)

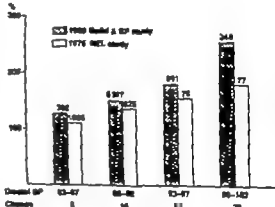


Fig 6 Comparison of mortality ratios by diastolic blood pressure category Men 40-49 (Brown & Singer 1976)

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	Male		Female	
	Treated	Control	Treated	Control
No. of cases	10	12	20	19
No. of strokes	0	4	3	3
No. of compl.	0	8	3	8

* $P < 0.025$

Table 3 Treatment vs nontreatment of hypertension: comparison of complications in the two sexes (Hamilton et al. 1964).

Table 5 Veterans Administration Cooperative Study Group: Classification of Morbid Events.

Diastolic BP	Treatment	Cerebro vascul.	CHD	Cardiac Other	Accel. hypert.	Renal	Other
115—129 mm Hg	Placebo	4	2	6	13	2	0
	Active	1	0	0	0	0	1
90—114 mm Hg	Placebo	20	13	11	4	3	5
	Active	3	11	0	0	0	6

The results after 6 years of observation encouraged the authors to recommend treatment of males with hypertension under the age of 60 regardless of other symptoms. The recommendations for women could not be made so firmly because of poor control of hypertension in the female group (Table 3).

In order to clarify the discussion on the value of active treatment of nonmalignant hypertension Freis and collaborators started a well controlled double blind study of male veterans in 1963 (10). The study included patients with diastolic blood pressure between 90 and 130 mm Hg with no signs of complications and whose ability to cooperate had been vouched for. After exclusion of nearly half the original material, 523 patients were randomized to active or placebo treatment. 143 patients with diastolic blood pressure of 115 or more were followed for 3 years (Table 4). After this time "it became apparent that the risk rate increased sharply and all patients in this group of high diastolic blood pressure were given active treatment. The 380 patients with lower blood pressure were followed for 5½ years and a better outlook for the actively treated was also demonstrated for this group of moderate hypertensives.

Subdividing the morbid events between different causes (Table 5) treatment seemed to have given significant protection against cerebrovascular accidents, cardiac insufficiency, accelerating hyperten-

Diastolic BP	Treatment	N	Dead	Other events
115—129 mm Hg	Placebo	70	4	10
	Active	73	0	0
90—114 mm Hg	Placebo	194	19	9
	Active	186	8	9

) nonterminating events

Table 4 Veterans Administration Cooperative Study Group: Mortality and morbidity in treated and nontreated patients.

sion and possibly renal failure in hypertensive patients of both grades. The lack of success in preventing future development of coronary heart disease may be an expression of the different ways in which elevated blood pressure acts as an accelerator of various pathological processes. One must bear in mind that in this study the mean age was over 50, and at this age the arteriosclerotic process may have reached a point of no return. Although this study carries many disadvantages in the selection of patients, it still represents the best support for an active attitude to hypotensive treatment.

As pointed out before there are obvious difficulties in drawing prognostic conclusions from clinical series of hypertensive patients. Only extensive follow-up studies of randomly selected people from the general hypertensive population can give a correct picture. Insurance applicants are by no means fully representative of the population in general. Comparisons within this selected group may however give some valuable information. At the recently held 12th International Congress of Life Insurance Medicine Brown & Singer presented a study (3) based on experiences from the New England Life Insurance Company 16 600 deaths were registered in half a million policies during the years 1954 to 1972 (Table 6). This period of 18 years represents a change from the time when the earlier mentioned Build & Blood Pres-

Patient Compliance

Hans Aberg

From the Department of Internal Medicine, University Hospital
Uppsala, Sweden

Abstract. Only a minority of all hypertensive individuals are well treated. In few either chronic disorders is the patient compliance to therapy such a challenging task. The reason is that a hypertensive patient is usually free from symptoms. Different methods can be used to study compliance. On the one hand we have simple interview methods and on the other we have sophisticated techniques for measuring the urine content of the drug. The best way of evaluating patient compliance over the years, however, is probably to study the number of drop-outs from follow-up or treatment failures with regard to reduction of the blood pressure. In a preventive trial on middle-aged men, over 3½-6 years of therapy we have had a drop-out rate of 8% and a mean blood pressure reduction of 30/18. In another study of drug behavior in a group of hypertensive individuals an acceptable level of compliance, measured as forgotten doses, was found in about 80% of the subjects. In spite of this rather high figure only a few subjects stated that they had received

information on their hypertension at the clinic. However, about half of the subjects did not want any more information. The problem of patient compliance to therapy has therefore many aspects, ranging from the psychological background just touched upon to more practically oriented matters such as dosettes etc. and I will review some of them.

Finally I would like to make some suggestions for improving both the patient's compliance and the doctor's attention to antihypertensive therapy.

A repeated finding in epidemiological studies has been that only a minority of hypertensive individuals are well treated. The figure given for men is about 20% (Table 1) in different parts of the Western world (1, 2, 3, 4, 5). It seems to be higher for women, but on the other hand studies in women (6) have been relatively few. It is a disappointing observation by many physicians that certain patients will not follow prescribed regimens regarding relatively simple medications which would

Table 1. The control of hypertension in different regions.

	Total	HYPERTENSIVES	
		Treated %	Adequately % treated
Uppsala			
♂ 49-59 y (ref. 1)	2322	50	29
♂ 60 y (ref. 2)	331	61	38
Östergötland			
♂ 47-54 y (ref. 3)	7452	39	22
♀ 36-46, 50-54 y (ref. 6)	1230	82	76
USA (ref. 4)	—	26	13
Australia (ref. 5)	—	45	15
Estimations			

- 11 Volhard, F & Fahr K. T. Die Brightsche Nierenkrankheit. Klinik Pathologie und Atlas, Julius Springer Berlin 1914

DISCUSSION

Göran Berglund

You mentioned the Fry study. We should keep in mind, that this study is a comparison between mortality rates in an economically well-off suburb of London and the total mortality rate in England. It may be so that these data are not representative for the whole hypertensive population.

Gösta Tibblin

What do you think about the better prognosis for high blood pressure among women than among men?

Ingvar Liljefors

We do not have any conclusive explanations for this, but women are also not comparable to men as far as other risk factors are concerned. One interesting finding in this connection is that cerebral vascular accidents are a more common cause of death in hypertensive women than in hypertensive men. This is shown in studies from Eliasch and others.

Gösta Tibblin

One explanation can be that the women are easier to treat. Certain experience points in this direction, but of course the disease may be different in women and men in some respect. The strange thing is, that up till now we have no controlled randomised study showing the benefit of treatment for women. That is the study we need to have.

Ingvar Liljefors

I would also like to mention the studies of Hamilton and Bechgaard, which show that women have a better prognosis than men.

Gösta Tibblin

Regarding the wording: Is it necessary to go on talking about "malign and benign" hypertension when even the benign hypertension is so malignant? Malignant hypertension—according to the prevailing nomenclature—is just one or two per cent of the whole hypertensive group. The prognosis is still bad in hypertension as a whole.

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Hans Åberg

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♀ 36–46, 50–54 y (ref. 6)	1230	82	76
US* (ref. 4)	—	26	13
Australia (ref. 5)	—	43	15

Estimations

not mean any marked change in their life-style. It is easier to accept non-compliance with recommendations to stop smoking or to lose weight, for example.

It was with the hope of improving this situation among others, that the "First International Congress on Patient Counselling" was held in Amsterdam on the 21st—23rd April 1976 with hypertension in focus.

The problem of patient compliance with therapeutic regimens is of course of considerable significance as regards chronic disorders such as hypertension and diabetes. Of these two hypertension gives the greater test of compliance, as failing therapy is not usually connected with the appearance of symptoms. However the problem of patient compliance is also encountered in short term treatment for an acute illness. Mattar et al. (7) have clearly demonstrated this in otitis media among 674 children attending an acute-diseases clinic.

The purpose of this paper is to review the subject from my own experiences as well as on the basis of the literature

HOW TO MEASURE COMPLIANCE

There are many different ways of evaluating a patient's compliance with a therapeutic regimen. One obvious way is to measure the serum level of the prescribed drug or the urine content of its metabolites. The latter method has been used by Lowenthal et al. (8) for example and in the famous VA study (9) where the compliance was evaluated in each patient during a run-in period. The urine was investigated for fluorescence as riboflavin was incorporated into the placebo tablet, and riboflavin has this ability. Only individuals showing such fluorescence were allowed to take part in the further studies.

However all these sophisticated methods can only be used in a limited number of patients and for a relatively short observation time. Such methods are of little help in chronic disorders, except in individual cases where serum levels might be examined to test the momentary reliability of the patient and/or the efficacy of the treatment.

It is important to keep in mind that all the elaborate ways of testing compliance might influence the final outcome of the test. Thus, it is clear that tablet counting will give a better result

if the patient is aware of what is going to happen. Also an interview might lead to better compliance, as the patient will hesitate to admit to doctor that he is careless about taking his. Nevertheless, an interview seems to be a fairly adequate method for evaluating patient compliance, particularly if it is conducted by a, other than the physician who has prescribed treatment (10, 11). This method also has the advantage of being easy to perform and to repeat, which is important in the lifelong treatment of such disorders as hypertension. We have carried out an interview study of patient compliance at a special clinic for hypertension. Defining the accepted level of compliance as "forgetting no more than one or a few doses a month" we found this level to be 81% (12). The interviews were not conducted by the prescribing physician.

One of the best ways of following the compliance to therapy over the years in treatment of a chronic disorder is probably to study the number of drop-outs from follow-up. If we exclude those individuals who have a natural reason for not attending follow-up (for example death, moving to another town etc.), the rest of the drop-outs must be considered as therapeutic failures due to non-compliance. There is also a certain, unknown number of patients who continue to visit their doctors but nevertheless have poor compliance in the prescribed treatment. In a study of middle-aged men treated for hypertension over a period of 3½—6 years, the number of drop-outs was 10 out of the original 106 subjects (13).

Another way to describe compliance to therapy in hypertension is to investigate how many hypertensive individuals there are who have a satisfactory blood pressure control. In other words, in how many of those on therapy has it been possible to reduce the blood pressure below a predetermined level of acceptance? In the study on middle-aged men mentioned above we have at present 9 subjects whose blood pressure has not reached a satisfactory level out of about 100 on medication. In Table 2 the mean blood pressure results are reviewed over the years in the subjects of this study.

FACTORS INFLUENCING COMPLIANCE

Pharmacological factors. As seen in Table 3 very few patients follow the prescribed interval between

Table 2. Blood pressures at screening and after treatment in a group of middle-aged men.

BLOOD PRESSURE					
Initial	1 y	2 y	3 y	4 y	5 y
174.5±19.0	150.1±20.0	146.3±16.8	143.9±19.2	141.8±16.2	145.0±17.5
111.8±7.7	97.3±8.6	95.6±9.2	93.7±9.9	92.6±9.4	94.2±8.7
(n=106)				(n=53)	(n=25)

Table 3. Dose intervals of interviewed hypertensives (n=81) at hypertension clinic. The circle denotes the recommended interval.

No. of doses/day	No. of patients. Time interval (in hours) between first and last dose.													
	5	6	7	8	9	10	11	12	13	14	15	16	17	18
2	1	—	1	1	1	6	4	⊙	1	1	—	—	—	—
3	—	—	1	3	2	6	6	3	5	1	1	⊙	—	—
4	—	—	—	—	—	—	3	7	3	7	3	1	—	⊙

Table 4. A review of the number of drugs taken concomitantly with the antihypertensive medication in the same group as in Table 3.

No. of antihypertensive drugs	No. of other drugs					Total
	0	1	2	3	4	
1	14	3	3	—	—	20
2	28	3	2	—	1	34
3	19	1	2	—	—	22
4	2	2	—	—	—	4
5	1	—	—	—	—	1
	64	9	7	0	1	81

doses (14). Naturally an exact interval between doses is only of importance if this is necessary for the effectiveness of the medication. A strict prescription should not be a purpose in itself. Another factor which should not be neglected is the concomitant use of drugs other than antihypertensive agents in a population with high blood pressure. In Table 4 the use of such drugs is reviewed in the previously mentioned material being interviewed. It is clear that if a patient has many other drugs to take in addition, this will complicate his antihypertensive therapy and this should be taken into account by the physician.

Further it is of value to combine, if possible, drugs that can be given at the same time interval. There are rational combinations of therapy that will decrease the number of pills and thus should improve the patient compliance. It should be unnecessary in this assembly to stress the importance of longacting drugs in lifelong therapy. Some stu-

dies have shown that the compliance is much higher when the patients have only one or at least a few doses each day (15, 16).

A major task for the pharmaceutical industry is of course to further develop the safety of drugs, i.e. freedom from serious side effects. The population in general is paying increased interest in side effects, probably due largely to the mass media.

Practical factors. Some authors have provided evidence that the use of different aids increases patient compliance to therapy. Thus, Haynes et al. (17) recently reported on a series that showed increased compliance as a result of visits by high-school graduates who checked their medication and arranged for the patient to be rewarded by being able to buy a sphygmomanometer and stethoscope at discount price. The value of dosettes should not be neglected and, these are in common use in Sweden nowadays. Another important task

not mean any marked change in their life-style. It is easier to accept non-compliance with recommendations to stop smoking or to lose weight for example

It was with the hope of improving this situation, among others, that the "First International Congress on Patient Counselling" was held in Amsterdam on the 21st-23rd April 1976 with hypertension in focus.

The problem of patient compliance with therapeutic regimens is of course of considerable significance as regards chronic disorders such as hypertension and diabetes. Of these two hypertension gives the greater test of compliance as failing therapy is not usually connected with the appearance of symptoms. However the problem of patient compliance is also encountered in short-term treatment for an acute illness. Mattar et al. (7) have clearly demonstrated this in otitis media among 674 children attending an acute-diseases clinic.

The purpose of this paper is to review the subject from my own experiences as well as on the basis of the literature.

HOW TO MEASURE COMPLIANCE

There are many different ways of evaluating a patient's compliance with a therapeutic regimen. One obvious way is to measure the serum level of the prescribed drug or the urine content of its metabolites. The latter method has been used by Lowenthal et al. (8) for example and in the famous VA study (9) where the compliance was evaluated in each patient during a run-in period. The urine was investigated for fluorescence as riboflavin was incorporated into the placebo tablet, and riboflavin has this ability. Only individuals showing such fluorescence were allowed to take part in the further studies.

However all these sophisticated methods can only be used in a limited number of patients and for a relatively short observation time. Such methods are of little help in chronic disorders except in individual cases where serum levels might be examined to test the momentary reliability of the patient and/or the efficacy of the treatment.

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if the patient is aware of what is going to happen. Also an interview might lead to better compliance as the patient will hesitate to admit to the doctor that he is careless about taking his drugs. Nevertheless, an interview seems to be a fairly adequate method for evaluating patient compliance particularly if it is conducted by a person other than the physician who has prescribed the treatment (10-11). This method also has the advantage of being easy to perform and to report, which is important in the lifelong treatment of such disorders as hypertension. We have carried out an interview study of patient compliance at a special clinic for hypertension. Defining the accepted level of compliance as "forgetting no more than one or a few doses a month" we found this level to be 81% (12). The interviews were not conducted by the prescribing physician.

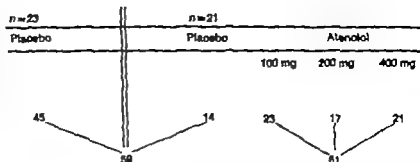
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Fig. 1 The number of side effects according to questionnaire in one group ($n=23$) taking a placebo for 16 weeks and another group ($n=21$) taking placebo for 4 weeks and Atenolol for 12 weeks at different dosages. (From Hansson et al. *Brit. Med. J.* 2: 367 1975)



than those attained by doctors in hospital as well as by private physicians.

The best result attainable seems to be a figure of about 80% for checking of all hypertensives in a given population after one year. This figure has been reached in a study comprising workers in Gimble's department store in New York City (27). This programme seemed to have all the ingredients to ensure perfect therapy for hypertension.

WHY DO PATIENTS DISCONTINUE ANTIHYPERTENSIVE THERAPY

Several different reasons have been given for discontinuing therapy. These are not identical in countries with different types of hospitals and social systems. Thus, in a study by Caldwell et al. in Detroit, U.S. 42 patients who had had a hypertensive crisis were questioned as to why they had discontinued their treatment (28). It is now several years since this study was performed, but the reasons given are nevertheless of interest. The majority stopped because they felt well (39%). In 33% the patients could not afford medical care. This is of course a figure which should not be reached in a system such as ours. A more remarkable reason however was that 24% were advised by a physician to stop their therapy. Another 14% lacked family support and only 7% discontinued therapy because of side effects. It seems that at least in our country there is a greater general awareness of the side effects of drugs. Therefore, a study such as that of Caldwell et al. is not only dependent on geographic and social circumstances but also on the time at which the study is performed.

In our country an increasing number of patients discontinue their drugs because of problems with alcohol, and people seem to be well aware of the danger of combining alcohol and tablets, at least

as long as they are not true alcoholics. It is surprising how often a patient asks if a tablet can be taken together with alcohol. Thus the patients must be well informed about this matter as that question is more often asked than what effect the antihypertensive drug has, or what the reason is for taking it.

CONCLUSIONS

In order to increase patient compliance to antihypertensive therapy some points should be stressed.

1. Start medication slowly to anticipate and overcome side effects.
2. Attempt to adjust the therapy to the patient's daily routine and make the dosage schedule feasible.
3. Give information but not necessarily all once. Individualize, and never frighten the patient.
4. Do not restrict the patient's life style with immediate recommendations to cease smoking and give up alcohol. If necessary tackle these problems later on.
5. Involve family members, particularly the wife or husband, in the treatment.
6. Encourage the patient again and again and be his counsel for the defence rather than the judge.
7. Last but not least, the education of doctors on hypertension should be improved.

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is to encourage each patient to identify any daily habit or ritual which he can connect with his anti-hypertensive medication. It would be an error for example to prescribe for a person who only eats twice a day that the drug be taken "three times a day with meals" What will the patient believe—is it important to take the drug three times a day or is it of no value if not taken with meals?

In a recent study it was found that the two factors having the greatest influence on compliance in discharged hospital patients were the complexity of the prescribed dosage schedule and the drug treatment before admission (18) Patients appear to board old prescribed medicines (19). It is difficult to know how to prevent this but hospital patients could be requested to hand in all their old medicines at the time of admission.

Some reports have also described programmes in which the patient was made responsible for the administration of his own drugs while under supervision in the hospital (20).

COMPLIANCE VERSUS INFORMATION

In our interview study of compliance we found that only a very small percentage of patients had received information on hypertension (Table 5). Even if this low figure could have been due partly to the fact that most of the patients were "old" hypertensives (had been hospitalized or at other outpatient clinics before) and supposedly were considered to be very experienced when starting at the Hypertension Clinic, it seems very unsatisfactory. However an interesting finding at the interview was that almost half of the patients did not want to have more information. Other studies have established the limited value of information at least in some patients. Thus Sacket et al. (21)

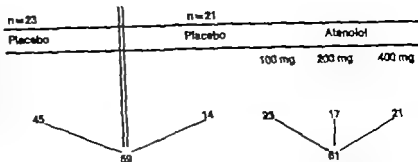
found that even "mastery learning" by means of an educational programme did not increase compliance. Failure of education to affect prevention therapy has also been shown recently by A. et al. (22). Are then non-compliance and patient apathy inevitable and all health education aimed at eliminating them of no value? Otherwise how can this lack of interest in information on such an important risk factor for future cardiovascular disease and death be interpreted? This constitutes patient psychology of a hitherto poorly studied type. It has been thought that there may be an unconscious desire to forget something (anti-hypertensive drugs) that will recall a future risk of suffering from some cardiovascular accident (23). This is strengthened by the fact that most individuals do not have symptoms from their hypertension (24). This lack of symptoms in hypertension makes it even more essential that the anti-hypertensive drug should not have side effects. It is common for patients under therapy to attribute all new unpleasant experiences to the drug even though "side effects" are also often experienced from placebo (Figure 1).

In order to handle this problem the previously mentioned negative attitude to information as well as the small impact of such information on a patient's compliance must be studied further (21). The most likely explanation for this lack of effect is that the information is poorly conveyed or given at wrong times. It is probably necessary to individualize such information and it should perhaps be given in small portions over a long period of time so as not to cause undue anxiety. Passive indoctrination of the patient should be avoided and an attempt should be made to get him to participate in his own treatment. Mc Kenney et al. (25) found that general information about high blood pressure was not an important element in promoting compliance. In their study a pharmacist was instructed to carry out tasks such as clarifying instructions, monitoring compliance and adjusting the medical regimen etc. and this was found to have an excellent effect. Their results thus demonstrate that other personnel than the doctor can be used as a source of information though the authors considered that the doctor should in fact carry out these tasks. Thus, Wilber (26) reported remarkably good follow-up results in a clinic run by nurses. These were better. In fact,

Table 5 Information received and wanted in an interviewed group of hypertensives.

Action of drug	6%
Side effects	8%
Diet	4%
No information	82%
Want more information	57%
Want no more information	43%

Fig 1 The number of side effects according to a questionnaire in one group (n=23) taking placebo for 16 weeks and another group (n=21) taking a placebo for 4 weeks and Atenolol for 12 weeks at different dosages. (From Hansson et al. *Brit. Med. J* 2: 367 1975.)



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DISCUSSION

Bengt Schersten

Are there any differences in compliance in relation to whether the patient is detected by screening or not or in relation to the presence or absence of symptoms?

Hans Aberg

I cannot give you any figures from our own studies, but I think these factors have been studied by Caldwell. His cases were detected in connection with hypertensive crises, and had all the symptoms you could wish for but the results after a short time were still poor.

Martin Ericsson

Our impression is that those patients who have a family background of hypertension are a risk group on which controls are worth performing.

Hans Aberg

There is no doubt that in those individuals who have a family history of hypertension or cardiovascular disease hypertension will tend to appear earlier at least will be discovered earlier. I think that they are also probably more adequately treated.

Konrad Saarnelli

It is often said that using twice a day dosage (one dose in the morning another in the evening) is superior with regard to compliance than using three doses daily. Can you confirm this impression and could you perhaps also comment on the

question of whether or not a once a day treatment schedule is superior to two daily doses?

Hans Aberg

Twice a day dosage is better than three times a day. Opinion is almost unanimous on this. This perhaps relates to the fact that patients do not need to take their medication to their job, which is difficult. On the other hand, I do not feel that there is such a big difference between twice a day and once a day dosage as there is between three times and twice a day although once a day dosage may well be better.

Anders Vedlin

Information on patient compliance can be collected from other areas. The severity of disease is the key factor in predicting patient compliance. In post myocardial infarction studies, 90 per cent of patients have complied with the prescribed dosage regimen.

In regard to the number of doses a day and its influence on compliance, this has been studied in several studies involving antibiotics. The results here indicate that the difference in compliance between single dose and twice a day dosage is small.

Hans Aberg

It is not right to compare rheumatic fever or myocardial infarction with hypertension. Hypertension is a chronic disorder without symptoms for most people and I think we need other studies than those you are referring to.

Gösta Tibblin

Maybe we can go on and talk a bit about information. You had a group which said that they do not want to have information. What is information in their mind? Perhaps they were just thinking about booklets? What we must do in those cases, is to change the behaviour of a person, an asymptomatic person who has no feeling of being sick. This is not so easy and I think we must discuss these problems and the methods needed to solve them. Perhaps study-groups and group therapy could be used for the treatment and information of these patients.

Hans Aberg

It is very important of course to give information and as you say I think we have to give it in other ways. I hand over a pamphlet on hypertension. It perhaps gives more relief for the doctor than it is for the patient. We have to individualize the information, to give it over a long time not all immediately and without causing them further anxiety.

Per Lund-Johansen

In regard to the question on what is a healthy and what is an unhealthy person, I think that some of us may have read or seen the play "Dr Knock, by

Romain, and perhaps remember his definition of a healthy person. "A healthy person is a person who does not know that he is sick." Bearing in mind Romain and Dr Knock, our definition today might be that a healthy person is a person who does not know his risk factors. But as long as we believe in preventive cardiology and as long as we believe that we can do something to postpone the complications and to prolong life in a reasonable way I think we of course have to do it.

Bengt Schersten

Could it be that the principles on which our patient care is based are in any way wrong? Perhaps we should not organize these antihypertensive clinics? Perhaps we should let the "Primary Care" take care of hypertensive patients? The continuity of the care is perhaps very important in getting patients to take the tablets.

Robert Griffiths

I imagine there might be some correlation between the number of tablets the patient is expected to take and the likelihood that he will not comply with taking them. Although we have all been told how bad combination medicines are, I think that, in the field of hypertension therapy this is one area where there might be a place for fixed combinations, if only because patient compliance is so terrible.

Hans Aberg

Well, I think it is important to discuss treatment with fixed combinations for hypertension. I think there might be a place for this, but obviously it is difficult to make a fixed combination which is adequate for many people.

Berni Hökfelt

To be provocative, fixed combinations to my mind, require standard patients and most people are not standard.

Haemodynamics in Essential Hypertension

Chairman Hans Dunér

Department of Internal Medicine Sabbatsberg Hospital Stockholm Sweden

Our knowledge of "haemodynamics in essential hypertension" has increased rapidly in recent years. 15—20 years ago essential hypertension, from the haemodynamic point of view was understood as a more or less fixed condition of increased peripheral vascular resistance and a normal or decreased cardiac output. Since then, we have obtained a more detailed and graduated picture of the role of the heart's activity and the condition of the peripheral vascular bed during the development of hypertension. The introduction of beta-receptor blocking drugs in the treatment of hypertension, has not only been of great importance from a therapeutic point of view but has also considerably increased our knowledge of haemodynamics.

In many clinics, beta-blockers are now the first line treatment for hypertension. It is interesting to see how quickly this development has occurred. When the first beta-blocker was launched at the beginning of the sixties, it was in the indication cardiac arrhythmia, and any blood pressure lowering effect was unknown. The first publication on the blood pressure lowering effect of beta-blockers came in 1964. We have here today one of the pioneers, Gustaf Schröder who together with Lars Werkö and at the same time as Frichard and Gillam reported this effect. At first there was a certain skepticism of the clinical value of beta-blockers for the treatment of hypertension. I will not go into details on the reasons for this. We can only see how accumulating evidence of the usefulness of beta-blockers as hypertensive agents has altered general opinion in this field. The first part of this session consists of two papers dealing with central and peripheral haemodynamics in essential hypertension, and I think it will be best to discuss these two papers together. The next part will deal with the effect of beta-blockers on

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haemodynamics in essential hypertension. It seems appropriate to discuss these two papers together as well, followed by four contributions submitted in advance to the discussion.

Per Lund Johansen from Bergen is the first speaker. As we all know he has carried out some very important studies on alterations in the pattern of haemodynamics with increasing age and on central haemodynamics in essential hypertension.

Central Haemodynamics in Essential Hypertension

Per Lund-Johansen

*From the Medical Department A University of Bergen School of Medicine
Bergen Norway*

INTRODUCTION

The arterial blood pressure is mainly determined by the cardiac output and the total peripheral resistance, and although it is a rather crude simplification, it is generally stated that $MAP = CO \times TPR$ (MAP = mean arterial pressure, CO = cardiac output, TPR = total peripheral resistance). The cardiac output is of course the product of the stroke volume (SV) and the heart rate (HR).

Until 1960 most studies on the haemodynamics in essential hypertension were done by heart catheterization and included usually small groups of subjects, mainly with established hypertension of long duration. The common finding was that the elevated systematic blood pressure was caused by an increased total peripheral resistance, while the cardiac output was normal so long as heart failure was not present.

CROSS-SECTIONAL STUDIES

Early phase About 15 years ago it was suggested that an increased cardiac output might be an important pathogenetic factor in early essential hypertension, and several studies from different parts of the world have appeared (reviews in references 15 and 19).

At rest, in the supine position, most investigators have found that in subjects below the age of 40 with a mean arterial pressure around 100 to 110 mm Hg (the time of the study the cardiac index is significantly higher than in controls, and the calculated total peripheral resistance not significantly different from controls (15). Studies at rest performed in the sitting position in young subjects with mild hypertension have also revealed high cardiac index (12, 18) but in subjects with borderline hypertension, who had high cardiac index in the supine position, the cardiac index be-

came normal when sitting (10).

Most investigators have found that the high cardiac output is due to an increase in the heart rate. It should be noted, however, that the cardiac output is not increased when it is related to the oxygen consumption of the body. This has been demonstrated in at least three studies (10, 12, 18). Thus there is no luxury perfusion in essential hypertension in contrast to the so-called hyperkinetic heart syndrome.

The mechanisms behind the high cardiac output, the high heart rate and the increased oxygen consumption in subjects with mild or labile essential hypertension are unknown. An increased activity in the sympathetic nervous system has been discussed but definite proof is still lacking.

In spite of the fact that the calculated total peripheral resistance falls within "normal" ranges in early essential hypertension, it could be argued that the total peripheral resistance is not normal, because if it were, it should have adapted to the high cardiac output with dilatation and thus be keeping the blood pressure normal. This is made clear when subjects are studied during muscular exercise. To avoid excessive rise in blood pressure, the resistance vessels must then dilate at least in some parts of the body. In a personal study on 137 untreated hypertensive males, age 17 to 59 years old, of whom 64 were below 40 years, it was found that during steady state exercise at 300, 600 and 900 kpm/min the cardiac index was no longer increased in any age group including the youngest (Figure 1 and 2). The reduced cardiac output during exercise was due to a reduced stroke volume. During exercise the total peripheral resistance was clearly increased in all age groups including the youngest. The rise in blood pressure was similar to the rise in controls when related

— normotensive
--- hypertensive

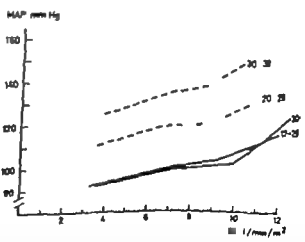
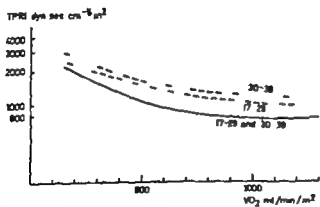
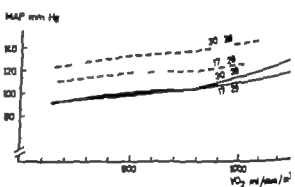
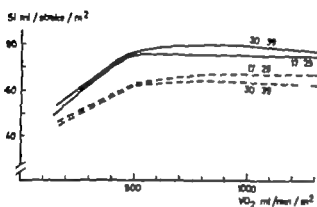
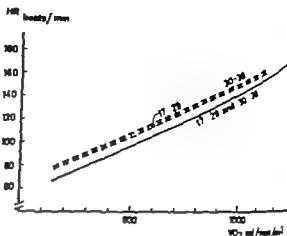
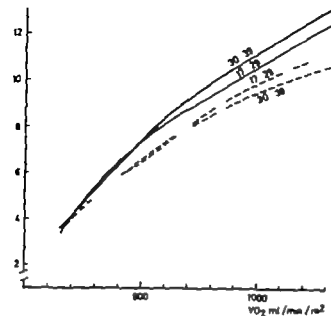
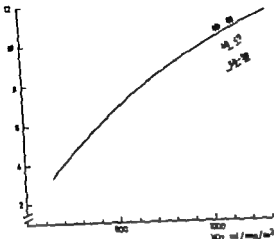


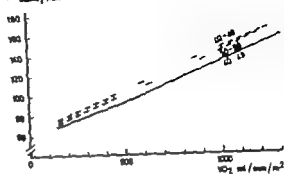
Fig 1 Haemodynamic changes at rest and during exercise in subjects with essential hypertension in WHO stage I aged 17-29 years and 30-39 years compared to normotensive controls. CI=Cardiac Index HR=Heart Rate SI=Stroke Index MAP=Mean Arterial Pressure TPRI=Total Peripheral Resistance Index.

VO_2 =Oxygen Consumption. Mean values from 22 normals and 64 hypertensives. (Reproduced from Omori, K. Kim, K. E. & Moyer J. H. (eds) Hypertension, Mechanisms and Management Grune & Stratton Inc. 1973 with kind permission).

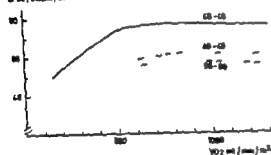
□ V_{O_2} ml/min/m²



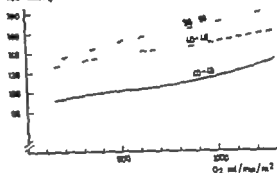
HR beats/min



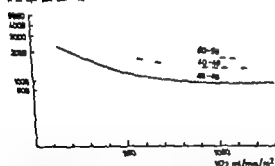
SI ml/min/m²



MAP mm Hg



TPRI dyn cm⁻⁵/m²



MAP mm Hg

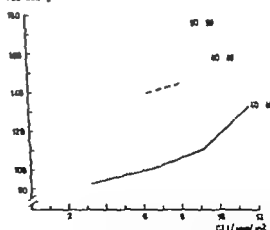


Fig. 2. Haemodynamic changes at rest and during exercise in subjects with essential hypertension aged 40-49 years (WHO stage I) and aged 50-59 years (WHO stage I or II) compared to normotensive con-

trols aged 40-49 years. CI=Cardiac Index, HR=Heart Rate, SI=Stroke Index, MAP=Mean Arterial Pressure, TPRI=Total Peripheral Resistance Index, V_{O_2} =Oxygen Consumption. (References as in figure 1).

the blood flow, the rise was steeper than in controls the steepest rise was seen in the oldest group. This was reflected in the calculated total peripheral resistance.

Most of these findings have been confirmed in exercise studies on the bicycle ergometer by other groups (1-18). Thus even if the calculated total peripheral resistance was not different from controls at rest it is evident from exercise studies that it is nevertheless already abnormal in subjects supposed to have "early" essential hypertension. Furthermore the exercise studies reveal that the heart pump function is subnormal due to a subnormal stroke volume and this is seen in subjects with mild essential hypertension already in their twenties.

Later phase In subjects with established essential hypertension of long duration, but without clinical heart failure the results from recent years are in agreement with earlier findings. At rest, the high arterial pressure is maintained by an increased total peripheral resistance in presence of a normal low cardiac index (1-12, 18). With increasing clinical severity of the disease the cardiac index and the stroke index tend to be very low and the total peripheral resistance very high. Essentially the same is found during muscular exercise. Exercise studies also demonstrate that in established essential hypertension the circulatory system might be hypokinetic even if no clinical signs of heart failure are present, and the arterio-venous oxygen difference is increased.

LONGITUDINAL STUDIES

Early phase Although the results from the cross-sectional studies make it very likely that the circulatory system in subjects with untreated essential hypertension should change from a high-flow normal resistance pattern towards a low-flow high-resistance pattern only longitudinal studies can tell if this will really happen. Until now systematic long-term studies have been lacking. In the following a preliminary report of a 10 year follow-up study in males with untreated essential hypertension will be given.

MATERIALS AND METHODS

The 1967 study included 77 hypertensives (17-66 years) and 33 normotensives (18-49 years) studied

cular exercise (12). Most of the 36 hypertensives below 40 years of age had mild hypertension: WHO stage I and did not start drug treatment. They were controlled clinically every year or every second year. In age group I (17-29 years, $n=19$) the mean value of the blood pressure recorded biannually was 150/92 mm Hg in 1967. Over the following 10 years 2 were lost abroad, 17 were alive healthy still in WHO stage I. 16 had been untreated and all but one (living far from Bergen) have been restudied haemodynamically.

In age group II (30-39 years old, $n=17$ all in WHO stage I) the mean value of the biannual pressure was 160/99 mm Hg when first studied. One subject died from lung and heart insufficiency. The remaining 16 patients are alive, all still in WHO group I. 13 have been untreated and they have all been restudied haemodynamically.

In the two oldest age groups the majority of the patients started drug treatment, and spontaneous occurring changes in haemodynamics could thus not be studied in these two groups. They will not be discussed further in this report.

Exactly the same methods were used in both studies. The subjects were studied at rest sitting and during steady state muscular exercise at 300, 600 and 900 kpm/min. Oxygen consumption was measured by Douglas bag and Scholander technique, heart rate by ECG, cardiac output by dye dilution method (Cardiogreen double determination in each situation), arterial blood pressure continuously by catheter in the brachial artery. The details have been published in 1967 (12).

RESULTS

A survey of the most important findings are shown in Figures 3-5 and Tables 1-3.

The oxygen consumption (VO_2) at rest had decreased from 171 to 157 ml/m^2 ($p<0.05$) in age group I but was almost unchanged in group II. During exercise there were only small changes.

The blood pressure showed remarkably small changes. During rest and low exercise levels there were no significant changes in systolic (SAP), diastolic (DAP) or mean (MAP) arterial pressures. Only during 900 kpm/min exercise there was a significant increase in MAP from 133 to 141 mm Hg in age group I and from 141 to 147 mm Hg in group II (Table 1).

Table 1 10 year follow-up study Mean arterial pressure (mm Hg).

1=First study 2=Restudy (Age at study 1)

		Rest		Work		(kpm/min)			
		Sitting		300		600		900	
		1	2	1	2	1	2	1	2
Age	Mean	112.3	114.7	120.5	123.6	122.9	126.0	133.1	141.0
17—	Sd	7.5	9.5	10.0	10.7	8.4	8.2	9.5	12.1
29 yrs	2-1	+2.4		+3.1		+3.1		+7.9	
n=13	p	Ns		N		Ns		<0.05	
Age	Mean	116.5	117.7	127.6	132.9	130.6	134.5	140.7	147.3
30—	Sd	9.5	10.7	12.3	10.0	12.2	8.6	15.5	12.4
39 yrs	2-1	+1.2		+5.3		+3.9		+6.6	
n=13	p	Ns		N		N		<0.05	

Table 2 10 year follow-up study Cardiac index (l/min/m²).

1=First study 2=Restudy (Age at study 1)

		Rest		Work		(kpm/min)			
		Sitting		300		600		900	
		1	2	1	2	1	2	1	2
Age	Mean	3.81	3.22	7.06	6.58	9.04	7.89	11.10	9.44
17—	Sd	0.53	0.83	1.03	1.20	0.83	0.68	1.03	0.88
29 yrs	2-1	-0.59		-0.48		-1.15		-1.66	
n=15	p	<0.01		N		<0.001		<0.001	
Age	Mean	3.64	2.81	6.82	5.82	8.49	7.48	10.41	8.96
30—	Sd	0.63	0.44	0.70	0.77	0.97	0.91	1.32	0.92
39 yrs	2-1	-0.83		-1.0		-1.01		-1.45	
n=13	p	<0.001		<0.001		<0.01		<0.01	

The cardiac index (CI) had decreased significantly in both age groups at rest as well as during moderate and severe exercise (Table 2). In the youngest group the reduction in cardiac index was only 0.48 l/min/m² (NS) at the lowest work level. In the oldest age group the CI was significantly reduced also at this low exercise level. In both age groups the reduction in cardiac index was associated with a significant decrease in stroke index (SI) at rest as well as during exercise.

The heart rate (HR) at rest and during exercise showed only small and insignificant changes in group I, but in group II there was a significant decrease in the resting value (from 80.4 to 71.3 beats/min).

The total peripheral resistance index (TPRI) had increased significantly both at rest and during exercise in both age groups (Table 3).

DISCUSSION AND CONCLUSION

The most important findings in the cross-sectional study from 1967 (12) have been confirmed by others (1, 10, 11, 17, 18, reviews in 13, 15, 19). In another previous study (14) it was found that in untreated subjects with mild hypertension no significant changes in the central haemodynamics could be detected over a period of one year.

In the 10 year follow-up study however it has been possible to demonstrate that significant changes in the central haemodynamics had indeed

Table 3 10 year follow-up study Total peripheral resistance index (dyn sec cm⁻⁵ m²).

1=First study 2=Restudy (Age at study 1)

		Rest		Work		(kpm/min)			
		Sitting		300		600		900	
		1	2	1	2	1	2	1	2
Age	Mean	2392	2971	1386	1531	1095	1285	965	1206
17—	Sd	284	574	168	206	111	125	93	133
29 yrs	2-1	+579		+145		+190		+240	
n=15	p	<0.001		<0.01		<0.001		<0.001	
Age	Mean	2532	3437	1533	1835	1242	1457	1091	1333
30—	Sd	480	684	142	270	152	190	143	220
39 yrs	2-1	+905		+326		+215		+241	
n=13	p	<0.001		<0.01		<0.01		<0.01	

Fig 3 Cardiac index (CI) mean arterial pressure (MAP) total peripheral resistance index (TPRI), heart rate (HR) and stroke index (SI) at first (1) and second (2) study. A 10 year follow-up study in untreated essential hypertension. Rest sitting.

10 year follow-up in untreated essential hypertension Hemodynamics at rest sitting
(1 first study 2 restudy Age at study 1) — mean value

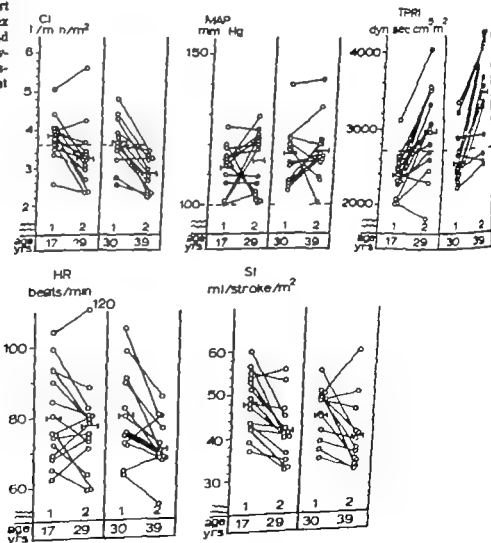


Fig 4 Cardiac index (CI), heart rate (HR) and stroke index (SI) at rest, sitting and during exercise at study 1 and 2. Mean values. Age group 30-39 years at study 1

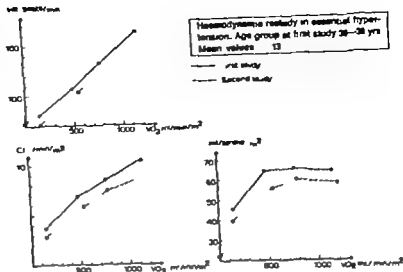
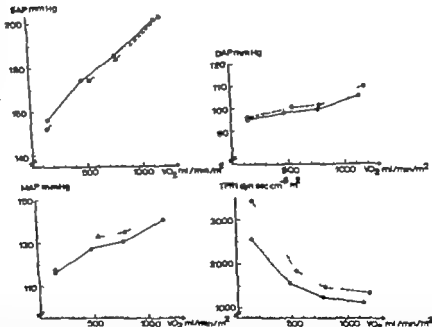


Fig 5 Systolic (SAP), diastolic (DAP) and mean arterial pressure (MAP) and total peripheral resistance index (TPRI) at rest and during exercise at study 1 and 2. Mean values. Age group 30-39 years at study 1



occurred in the youngest group the increased VO_2 had fallen close to the value in age group II (10 years ago). The increased heart rate persisted, but in age group II (now being 40-50 years old) the resting heart rate had decreased. In both age groups the reductions in SI and increases in TPRI were unimpressive and very consistent. Thus in spite of the fact that the subjects seemed to have tolerated their hypertension well—without any

overt clinical complications—the haemodynamic study showed that their cardiovascular system had deteriorated more than expected from aging alone. Several cross-sectional studies in healthy normotensive subjects have demonstrated that aging from the twenties to the forties involves no or only negligible changes in the cardiac performance (2, 4, 8, 9). This is in clear contrast to what happens in the hypertensives. No systematic studies

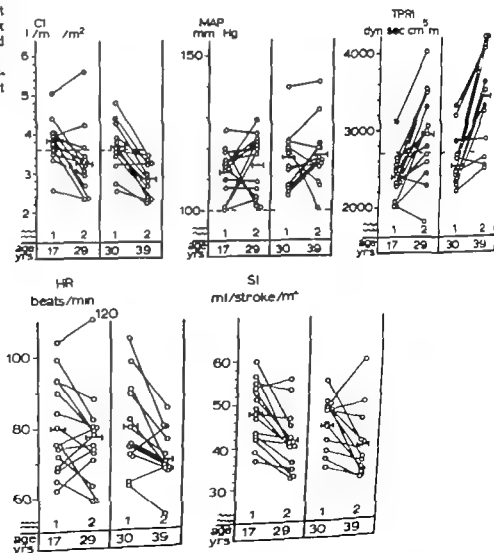
Table 3 10 year follow-up study Total peripheral resistance index (dyn sec cm⁻³ m²).

1=First study 2=Restudy (Age at study 1)

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10 year follow-up in untreated essential hypertension Hemodynamics at rest sitting (1 first study 2 restudy Age at study 1) — mean value



Peripheral Haemodynamics in Essential Hypertension

Ramon Sivertsson

*From the Department of Clinical Physiology Östra Sjukhuset (East Hospital)
Göteborg, Sweden*

Abstract. Previous studies have indicated the existence of haemodynamically important structural vascular changes in patients and animals with hypertension.

Possible pathophysiological implications of these changes are discussed.

Two studies dealing with the question of reversibility of the structural changes on long-term blood pressure lowering therapy are described. The results indicate that the changes are partially but not completely reversible.

STRUCTURAL VASCULAR CHANGES IN HUMAN HYPERTENSION

In patients with established hypertension blood pressure is elevated because vascular resistance is increased. Cardiac output is normal. Haemodynamic studies on patients with untreated essential hypertension indicate that resistance is increased not because of high tone in the smooth muscle cells of the resistance vessels but rather because of structural changes in these vessels (1, 2, 3, 4). Thus vascular resistance in the hand at maximal vasodilatation was increased to a considerable degree in the patients compared to the controls (4) in fact more than the mean blood pressure (69 per cent and 46 per cent respectively). Since resistance in this study was measured under almost complete relaxation of the vascular smooth muscle cells the difference in resistance can only be explained by structural difference between the two vascular beds. Because of this finding and other haemodynamic findings in combination with the observations done by the pathologists (5, 6) it was concluded that in established essential hypertension resistance is mainly increased because of a structural change in the resistance vessels. This change implies an increased wall thickness in relation to

lumen and the thickened wall encroaches upon the lumen also at maximal dilatation. The wall thickening, which seems to involve the resistance vessels of most vascular beds more or less, is partially due to smooth muscle hypertrophy partially to increased amounts of other wall components, e.g. collagen. Corresponding changes are also seen in the bigger arteries.

HOW DO THE VASCULAR CHANGES ARISE?

Animal experiments show that structural changes can be secondary to the blood pressure elevation (7). Although it cannot be excluded that the vascular changes are primary in some patients (genetically based tendency to smooth muscle hypertrophy), they are most likely secondary in the majority of patients with essential hypertension. A primary "triggering" factor (e.g. a neurogenic, a renal or a hormonal one) is thus needed.

PATHOGENETIC IMPLICATIONS OF VASCULAR CHANGES

If the changes are secondary to blood pressure elevation what is then the role of these changes in hypertension?

1. When established the vascular changes potentiate the primary blood pressure elevation.
2. They transform a labile hypertension to a stable one. Before the vascular changes are established blood pressure, e.g. in neurogenic hypertension is supposed to be normal in the periods between the neurogenic pressure peaks, but after the vessels are changed this "resting" pressure is also elevated.
3. If the changes are irreversible or only partially reversible they will constitute a permanent blood pressure elevating factor which remains even when the primary cause is eliminated.

similar to this have been presented. However one study in treated subjects taken off therapy for a short period of time (3) and another study of shorter duration (5) did show similar trends as those in the present study. It is therefore reasonable to conclude that the 10 year duration of hypertension was responsible for the far greater alterations in the central haemodynamics than would be expected from aging alone.

What is the mechanism behind the decrease in the SI and in the increase in the TPRI? What is the meaning of these changes with respect to the progress of the hypertensive process? This has to be answered by future studies. However these functional alterations show a remarkable similarity to what is seen in the spontaneously hypertensive rats (SHR) when the time factor is expressed as a fraction of life duration (6, 7, 16, 20). In the SHR a reduced SI is a very early abnormality and so is the increase in the TPRI. Furthermore in the normotensive rat the pump function seems to be little altered by time. In the rats it is of course possible to study the morphological and biochemical abnormalities behind the functional changes in the central haemodynamics. It is beyond the scope of this article to discuss these findings in detail, but largely all these findings taken together seem to fit well with Folkow's theory about the restructuring of the high pressure compartments (7). Thus the "vicious circle" seems to be operating also in man but slowly and therefore hard to study. Another crucial question is of course whether these changes are reversible and at what stage antihypertensive therapy should then be started.

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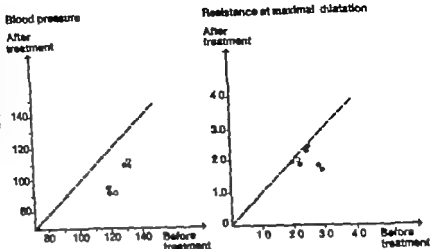
similar to this have been presented. However one study in treated subjects taken off therapy for a short period of time (3) and another study of shorter duration (5) did show similar trends as those in the present study. It is therefore reasonable to conclude that the 10 year duration of hypertension was responsible for the far greater alterations in the central haemodynamics than would be expected from aging alone.

What is the mechanism behind the decrease in the SI and in the increase in the TPRI? What is the meaning of these changes with respect to the progress of the hypertensive process? This has to be answered by future studies. However these functional alterations show a remarkable similarity to what is seen in the spontaneously hypertensive rats (SHR) when the time factor is expressed as a fraction of life duration (6, 7, 16, 20). In the SHR a reduced SI is a very early abnormality, and so is the increase in the TPRI. Furthermore, in the normotensive rat the pump function seems to be little altered by time. In the rats it is of course possible to study the morphological and biochemical abnormalities behind the functional changes in the central haemodynamics. It is beyond the scope of this article to discuss these findings in detail, but largely all these findings taken together seem to fit well with Folkow's theory about the restructuring of the high pressure compartments (7). Thus the "vicious circle" seems to be operating also in man, but slowly and therefore hard to study. Another crucial question is of course whether these changes are reversible, and at what stage antihypertensive therapy should then be started.

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Fig. 2. Mean arterial systolic blood pressure and calf blood flow resistance at maximal vasodilatation before and after 6 months of antihypertensive treatment (propranolol or atenolol and hydralazine) in 12 patients with essential hypertension.



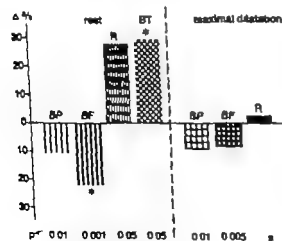
MEFRUSIDE 25 mg daily 6 weeks n=23



MEFRUSIDE 25 mg daily 6 months n=23



ATENOLOL 100-400 mg daily 6 weeks n=17



ATENOLOL 100-400 mg daily 6 months n=18

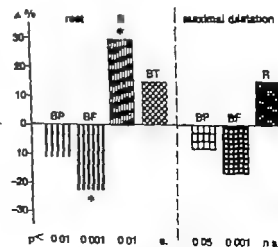


Fig. 3. Change in mean arterial systolic blood pressure (BP), calf blood flow (BF) and blood flow resistance (R) at rest and under vasodilatation after 6 months treatment with mefruside in twenty-three hypertensive patients (upper panel) and with atenolol in

eighteen patients (lower panel). Vascular tone (BT), i.e. resistance at rest divided by resistance under vasodilatation, is also given. The asterisk (*) indicates significant difference between the mefruside and atenolol groups.

- 4 Corresponding changes in the big arteries are probably responsible at least partially for the baroreceptor resetting in hypertension, the thickened arterial wall being less distensible than the normal one. Thus a higher pressure is needed to activate the stretch receptors involved in the barostatic reflex.
- 5 Recently a long term barostatic function has been ascribed to the kidney (8). The kidney barostat is probably also reset by structural vascular changes, localized to the renal vessels.

REVERSIBILITY OF VASCULAR CHANGES ON LONG TERM BLOOD PRESSURE LOWERING THERAPY

From what is said above it is evident that the question of reversibility of vascular changes has a fundamental bearing to the pathophysiological role of the changes. Studies on animals (spontaneous and renal hypertensive rats) indicate that hypertensive vascular changes can be prevented if blood pressure lowering therapy is started in the new born animal (9). They can be completely reversed if pressure normalisation is initiated early (7), but if treatment is not begun until the animal is middle-aged or old then the changes are only partially reversible (9). To study the possible reversibility of hypertensive vascular changes in man we (10) examined 13 patients and 13 matched controls before and after five years of antihypertensive treatment in the patient group. Intraarterial blood pressure and hand blood flow was studied. Figure 1 shows the difference in mean blood pressure between patients and controls before and after treatment. This difference is considerably reduced by the therapy (from 46 per cent to 10 per cent). The corresponding difference in resistance at maximal dilatation is also significantly reduced after treatment (from 46 per cent to 14 per cent). These experiments thus indicate that the structural changes in the hand blood vessels are at least partially reversible.

To elucidate this question further we have also studied calf blood flow and indirect arm blood pressure in 54 patients with essential hypertension (WHO stage I II) before and after six months of blood pressure lowering therapy (Figures 2 and 3). Twelve patients have been treated with propranolol or alprenolol and hydralazine, eighteen with atenolol and twenty-three with mefruside. Maximal

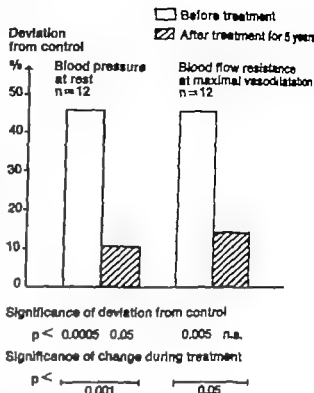


Fig 1 Intra-arterial blood pressure and hand blood flow resistance at maximal vasodilatation expressed as the percent deviation of the hypertensive group from the control group before and after antihypertensive treatment for 5 years.

dilatation was induced by a combination of arterial occlusion in the leg and calf muscle work until exhaustion. Under conditions of vasodilatation none of the drugs changed calf blood flow resistance significantly.

There may be several explanations for the discrepancy between the two follow up studies. The duration of treatment is much shorter in the latter one where calf blood flow is examined than in the first one. Furthermore as the calf is exposed to a higher pressure than the hand because of man's upright position the vascular changes in the calf may be of a different nature from those in the hand. Like vascular changes in more severe and long standing animal hypertension the vascular changes in the calves of hypertensive patients may show only a limited reversibility (9) because of an extensive collagen invasion in the arteriolar wall.

It can thus be concluded that vascular changes as reflected by blood flow resistance at maximal dilatation in patients with established essential hypertension seem to be partially but not completely reversible on long term blood pressure lowering therapy. Regional differences may exist.

Ramon Sverrisson

I do not think so.

Harald Eliasson

We obtained the same results as you when we re-studied Varnumskas hypertensive case material from 1955 (Scand. J. Clin. Lab. Invest. 1955 suppl. to vol. 7). We found a decrease in cardiac output along with time. (Hypertoni och aterosklerosfrågor Sällskapsdokumentet 1970.)

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DISCUSSION

Lennart Hansson

I would like to ask Per Lund Johansen. Have you also re-examined the normal volunteers, whom you had ten years ago and if so did they show the same change in haemodynamics as the hypertensives? Secondly I think it is important, as you stressed that the methods in the first and second study should be identical. Did you in any way check this and if so how did you do it?

Per Lund Johansen

The answer to the first question is that I have so far not studied the normotensive controls. Nevertheless I think that it is possible to draw some conclusions about the differences between the hypertensives and the normotensives. In cross-sectional studies in normotensives, there is a lot of data from USA Canada and I think from Sweden, showing that, as long as the blood pressure is normal, very little actually happens to the cardiac output, the stroke volume and the calculated resistance over the period from 20 up to 45 years of age. But, of course I agree with you that it would have been very interesting if the normotensive subjects had been studied as well.

My answer to your second question is that all patients were out-patients, and they were studied at exactly the same time of day with exactly the same type of equipment, following exactly the same experimental procedure in both the first and the second study

Bengt Pernow

I would like to comment on doctor P Lund-Johansen's findings on cardiac output. In the younger age-groups at least, there was generally found to be a high cardiac index, and it was interesting to see that this high cardiac output ran in parallel to a large oxygen uptake while the arterio-venous oxygen difference was normal. This speaks in favour of a higher metabolic rate in these patients, and obviously they were not what is termed "hyperkinetic". I would like to define hyperkinetic circulation as a situation with a low arterio-venous difference. I think that a true hyperkinetic state, which is frequently referred to in literature, is extremely rare in fact, and the reason for the high cardiac output in your cases was, exactly as you suggested that they had a high metabolic rate. Secondly you found when you studied these patients again ten years later that the cardiac index had decreased. I would like to ask you. Was there a decrease in the oxygen uptake at the same time? In other words, was the decrease of the cardiac index due to a decrease in metabolic rate or was it due to structural changes in the heart, changing the heart muscle compliance?

Per Lund-Johansen

I agree with your comments about hyperkinetic circulation and also with your definition of this term. My answer to your second question is that in the youngest age-group the oxygen consumption also decreased at rest. However during muscular exercise there were no significant changes in the oxygen uptake and there was still a decrease in the stroke volume. I therefore think it is possible that the reduction in the stroke volume could have something to do with structural changes in the myocardium, this is speculation of course, but it is in line with results of studies on spontaneously hypertensive rats.

Bengt Schersten

Is there a critical level of blood pressure which must be passed before structural changes can be expected in the vessels?

Effects of Beta Adrenoceptor Blocking Agents on Haemodynamic Parameters

Lennart Hansson

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Abstract. A review is given of some of the important studies dealing with the haemodynamic effects of beta-adrenoceptor-blockade in the treatment of hypertension.

Emphasis is put on the haemodynamic changes that occur during long-term beta-adrenoceptor blockade and comparisons are made between acute and long-term effects, particularly as regards the change of total peripheral vascular resistance.

As hypertension is a multifactorial disease, one should not expect to find one single mechanism by which the antihypertensive mode of action of beta-adrenoceptor-blocking agents could be explained. However irrespective of whether a humoral or a neurogenic or other possible mechanism is favoured, it is axiomatic that such mechanisms would have to influence blood pressure through changes of either cardiac output or total peripheral vascular resistance (or both). For this reason the haemodynamic changes will always be of important

The topic of combined treatment of hypertension using a beta-adrenoceptor-blocking agent in conjunction with a diuretic and the haemodynamic effects thereof is briefly discussed, and finally some data are presented on the effect of beta-adrenoceptor-blockade on regional haemodynamics.

The clinical usefulness of beta-adrenoceptor blocking agent in the treatment of elevated arterial pressure is well documented (12, 32, 33, 42). Following the first clinical reports on the antihypertensive effect of pronethalol (30, 36) and propranolol (31) in 1964, blood pressure lowering effect has been claimed for almost all other agents of this category.

However the mechanism responsible for the reduction in arterial pressure still remains to be fully explained. Since most forms of established hypertension are characterized by a normal cardiac output and an elevated total peripheral vascular resistance (9) and since acute administration of a beta-adrenoceptor blocking agent is known to increase systematic vascular resistance (7, 26, 41) the first reports of the antihypertensive effect were surprising.

As blood pressure is a haemodynamic parameter directly dependent on cardiac output and total peripheral vascular resistance it is obvious that any change of blood pressure will have to rely on a change of either cardiac output or total peripheral resistance (or both). For this reason, suggested mechanisms involving factors such as the renin-angiotensin-aldosterone system (3), interference with presynaptic neurons or membrane-stabilizing (local anesthetic) properties of these compounds (5, 6) will not be dealt with in detail in this paper since, even if such mechanisms are operable and of practical importance, their final influence on arterial pressure would have to operate through changes of either cardiac output or total peripheral resistance.

This should not be understood to mean that one single factor can be expected to explain the antihypertensive effect of beta-adrenoceptor blocking agents. On the contrary as essential hypertension is most probably multifactorial in origin, it should be expected that the blood pressure lowering effect of beta-adrenoceptor blocking agents can be obtained through various mechanisms.

HAEMODYNAMIC STUDIES

There is an abundance of haemodynamic studies

Tarazi and Dustan could also demonstrate that the change of mean arterial pressure was significantly correlated to the change of total peripheral resistance but that the change of blood pressure was not correlated to the change of cardiac output (37).

These haemodynamic differences between the acute and longterm effects of beta-adrenoceptor blockade in hypertensive patients have been confirmed by Hansson (11). The re-adjustment of total peripheral vascular resistance that occurs during prolonged treatment is illustrated in Figure 1. That a reduction of cardiac output per se is not sufficient to cause a fall of arterial pressure in the absence of a re-adjustment of total peripheral resistance is illustrated in Figure 2. There "non-responders" who actually showed a 5% increase of mean arterial pressure during prolonged treatment with propranolol had a reduction of cardiac output, which was actually more pronounced than that seen in the responders. However total peripheral resistance remained high in the non-responders also during prolonged beta-adrenoceptor blockade (11). It should be mentioned that the percentage reduction of stimulated plasma renin activity was of the same order in both groups of patients (14).

It can thus be concluded that a re-adjustment of total peripheral vascular resistance occurs during prolonged beta-adrenoceptor blocking therapy in those hypertensive patients who respond to the treatment. The underlying mechanism for this re-adjustment is still not clarified. A change of baroreceptor activity seems logical and has been suggested (11, 33). However whether this alteration of baroreceptor mediated vasoconstrictor nerve activity is associated with pre-synaptic beta-adrenoceptor blockade as recently suggested by Laager (22) or whether other metabolic events are of importance remains to be elucidated.

The demonstrated haemodynamic alterations during long-term beta-adrenoceptor blockade are of practical importance. Thus, during the early years of using beta-adrenoceptor blocking agents in the treatment of hypertension, it was often suggested that this kind of treatment should be reserved for individuals with high heart rates or high cardiac outputs. This seemingly logical approach—logical in view of the evident and easily demonstrated cardiac effects of these agents—can-

not be justified after an analysis of available data. Thus, there is no correlation between pre-treatment levels of heart rate or cardiac output and blood pressure reduction obtained during beta-adrenoceptor blockade (2, 15, 37). Even patients showing marked tachycardia and marked elevation of cardiac output do not invariably show a reduction of their elevated arterial pressure in spite of the fact that tachycardia and elevated cardiac output could be controlled by beta-adrenoceptor blockade (17).

It can thus further be concluded that the presence of high heart rate or high cardiac output (hyperkinetic circulation) in the pre-treatment situation will not per se guarantee that blood pressure will be controlled when a beta-adrenoceptor blocking agent is administered.

On the other hand it may be argued that beta-adrenoceptor blockade instituted in the early phase of hypertension—when cardiac participation supposedly is of great importance (19, 20)—seems to be effective in preventing a rise of blood pressure in spontaneously hypertensive rats, whereas no effect could be demonstrated when this treatment was given during later stages (8). However also in this situation objections could be raised. Thus, it has been shown that propranolol cannot prevent development of hypertension during electrical stimulation of the stellate ganglion in conscious dogs, indicating that cardiogenic hypertension may develop in the presence of beta-adrenoceptor blockade (23).

Recent haemodynamic studies

During the last few years a number of studies have been published, which deal with haemodynamics in hypertensive patients before and during treatment with various beta-adrenoceptor blocking agents. Of great interest are the studies made by Lund-Johansen, who has studied a number of beta-adrenoceptor blocking agents and their effect on haemodynamic parameters, during rest and physical exercise. Usually these studies have been performed before and following one year of treatment with the respective drugs. In a comparison of alprenolol, tenolol, metoprolol and timolol

statistically significant reduction of mean arterial pressure (11–18%) was found with all the above agents. Heart rate was also significantly reduced in all groups in this study. However due

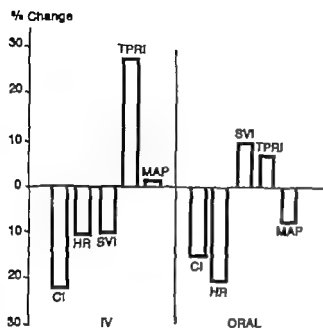


Fig 1 Changes of haemodynamic parameters following acute and prolonged beta-adrenoceptor blockade with propranolol in hypertensive patients. CI=Cardiac Index HR=Heart rate MAP=Mean Arterial Pressure TPRI=Total Peripheral Resistance Index.

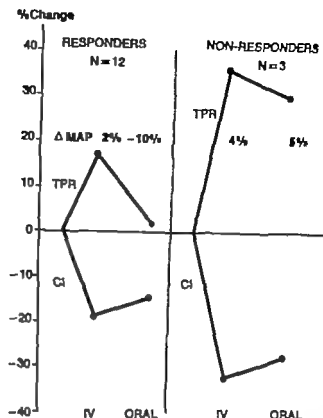


Fig 2 Changes of haemodynamic parameters in 12 patients showing a reduction of blood pressure and 3 patients showing no reduction of blood pressure during prolonged treatment with propranolol. CI=Cardiac Index, MAP=Mean Arterial Pressure, TPRI=Total Peripheral Resistance Index.

with several different beta-adrenoceptor blocking agents in the literature. It is beyond the scope of this paper to cover them all. However a few selected papers which have contributed to our understanding of the haemodynamics of these agents will be reviewed. The haemodynamic studies with beta-adrenoceptor blocking agents in hypertensive patients performed at the Cleveland Clinic Research Division are undoubtedly of prime interest in this respect. Thus, in 1968 Urych et al published reports on acute intravenous administration of propranolol to normotensive and hypertensive subjects (41). A significant reduction of cardiac output was demonstrated. However due to a compensatory increase of calculated total peripheral vascular resistance the mean arterial blood pressure did not change (41). This finding was not surprising in view of previous results with acute administration of a beta-adrenoceptor blocking agent. However the Cleveland Clinic group at the same time published reports

on the long-term effects of beta-adrenoceptor blockade in a small number of hypertensive patients (10). In this study they reported the paradoxical finding that blood pressure was decreased in spite of a chronic reduction of cardiac output.

Tarazi and Dustan have later demonstrated the striking difference between the acute and chronic haemodynamic effects of beta-adrenoceptor blockade in hypertension. Following acute administration of propranolol, they confirmed earlier observations that heart rate and cardiac output were reduced significantly but due to a compensatory increase of total peripheral vascular resistance blood pressure did not change in this situation. However during repeated haemodynamic examinations in the same patients a significant reduction of mean arterial blood pressure was found due to the fact that total peripheral resistance was re-adjusted down to the initial level in the presence of a maintained reduction of cardiac output (37).

Turner and Dornan could also demonstrate that the change of mean arterial pressure was significantly correlated to the change of total peripheral resistance but that the change of blood pressure was not correlated to the change of cardiac output (37).

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to differences in stroke volume total peripheral resistance did not change uniformly. Thus, in the timolol group total peripheral resistance at rest remained significantly elevated after one year whereas this parameter was "normalized" during treatment with alprenolol, atenolol and metoprolol (24).

Studies with pindolol before and after 8 weeks of treatment have also demonstrated that statistically significant reductions of systemic arterial pressure are obtained (1). In agreement with other studies significant reductions of heart rate and cardiac output were observed, whereas no significant changes of stroke volume or total peripheral resistance were seen (1).

Combined treatment with vasodilators

As most forms of established hypertension are characterized by normal cardiac output and elevated peripheral resistance (9) a vasodilator would appear as the most logical remedy. However due to compensatory mechanisms elicited through the baroreflex mechanism treatment with a vasodilator as the sole agent is rarely successful. Clearly the combined use of a vasodilator and a beta-adrenoceptor blocking agent would appear to solve the problem of reflexogenic increase of sympathetic tone particularly as regards stimulation of the heart. Several clinical applications of such combinations have also proved to be useful (13, 21, 35). The haemodynamic value of adding a beta-adrenoceptor blocking agent to a vasodilator drug therapy has been demonstrated e.g. as regards alprenolol and dihydropyridine (35) or tolazoline and prazosin (25).

The additional reduction of blood pressure when a beta-adrenoceptor blocking agent is added to a vasodilator drug regime has been claimed to be dependent on a renal suppressing action of the beta-adrenoceptor blocker in this situation (29). However others have not been able to establish a correlation between the humoral and haemodynamic changes during combined therapy (38).

Regional haemodynamics

It is easy to overlook the fact that total peripheral resistance is a calculated parameter depending on the exact measurements of arterial pressure and cardiac output. Total peripheral resistance thus reflects the sum of resistance patterns

in all vascular beds of the body. Clearly directionally opposite changes of resistance may occur in different vascular beds, the effects of which will eliminate themselves as regards influence on total peripheral resistance. Unfortunately few vascular beds have been studied during the influence of beta-adrenoceptor blocking agents.

As regards cerebral blood flow it has been shown in monkeys that cerebral blood flow is relatively less reduced than blood flow in other vascular beds (27). The effect of pindolol on forearm blood flow (mainly a muscle vascular bed) shows no reduction of blood flow and no change of resistance at rest. However during physical exercise a significant reduction of forearm resistance was found (1). Similar studies with propranolol and atenolol show significant reductions of systemic arterial pressure but also significant reductions of blood flow at rest in the calves (mainly a muscle vascular bed) (16). The reduction of blood flow to the legs during exercise and rest due to treatment with propranolol has been found to be directly related to a reduction of arterial pressure and thus resistance in this vascular bed does not increase during chronic beta-adrenoceptor blockade (39, 40). This is in agreement with studies *in vivo* showing a reduction of muscle blood flow (28). Moreover it has been reported that glomerular filtration rate is decreased in hypertensive patients during long term beta-adrenoceptor blockade (18), although this finding has not been seen in all patients by other investigators (38). Liver blood flow has been found to fall more than would correspond to the reduction in systemic arterial pressure during prolonged beta-adrenoceptor blockade thus indicating an increase of vascular resistance in the liver (4, 39). This reduction of blood flow to the liver could have implications for the metabolism of some of the beta-adrenoceptor blocking agents themselves.

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Haemodynamic effects of pindolol in hypertensive patients

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Abstract. Five men and five women, mean age 48 years, with hypertension in stages I or II of the WHO classification, were given peroral treatment with pindolol.

The pindolol treatment led to a significant decrease in the systolic and diastolic blood pressure, at rest and during work both after 2 months and after 16 months treatment.

Three mechanisms seem to be involved in the antihypertensive effect of pindolol: 1) a negative chronotropic effect on the heart, 2) a decrease in peripheral vascular resistance and 3) an increase in venous capacitance affecting the venous return.

Comparison of the results after 2 and after 16 months of treatment suggests that a decrease in cardiac output is an early mechanism in the lowering of the blood pressure, while a decrease in vascular resistance seems to be more important after long-term treatment with pindolol.

Although beta-adrenoreceptor blocking agents have been widely used in the treatment of hypertension for more than ten years, the mechanisms by which the antihypertensive action is achieved are not yet fully explained. This is probably partly due to the fact that a uniform mode of action of betablockers does not exist in the multifactorial disease which essential hypertension constitutes. Furthermore it has been repeatedly shown, that betablockers influence both cardiac output and total peripheral resistance, the two haemodynamic variables mainly responsible for the blood pressure level. In addition, the effect of the betablocking agents on haemodynamics is entirely dependent on

the pharmacological characteristics of the particular preparation, including degree of selectivity, intrinsic activity and quinidine-like effect.

Pindolol (Vlsken® Sandoz) is a potent beta-adrenoreceptor blocking agent with a weak quinidine-like effect, a receptor stimulating activity but no effect on alpha-adrenergic receptors. The hypotensive effect of pindolol is well documented (2, 8, 10, 12).

MATERIAL AND PROCEDURE

The material consists of ten patients, 5 women and 5 men with essential hypertension (stage I and II according to the WHO classification). The mean age of the patients was 47.8 years (range 31–61) at the start of the study. The blood pressure criteria for hypertension was a systolic pressure of not less than 15 mm Hg and a diastolic pressure not less than 105 mm Hg. Patients with substantial disorders in addition to hypertension such as latent or manifest cardiac insufficiency, bronchial asthma, renal insufficiency or diabetes, were excluded. No signs of organic cardiovascular changes were present in eight of the patients (stage I); the other two had a slightly enlarged heart volume but no other signs of cardiovascular changes (stage II).

At the start of the study none of the patients had any hypotensive medication. The initial blood pressure measurements, confirming the diagnosis of hypertension in the individual case, were made on three occasions separated by intervals of about one week. The blood pressure was measured indirectly on the left upper arm after 5 min rest in the supine position. Thereafter a run-in placebo period of 4 weeks duration started with a blood pressure recording after 2 and 4 weeks. The placebo period ended with a haemodynamic examination.

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		Control	2 months	16 months	Diff 2-16 months
Mean arterial blood pressure mm Hg	Rest	140	-23	-27*	
	Exercise	139	-29*	-33**	
	After exercise	134	-21	-23	
Heart rate beats/min	Rest	74	- 8*	- 7*	
	Exercise	119	-18**	-18*	
	After exercise	84	- 9*	- 7*	
Cardiac output l/min	Rest	5.5	- 0.7*	- 0.4	
	Exercise	10.4	- 1.4**	- 0.4	
	After exercise	6.1	- 0.3	± 0	
Stroke volume ml	Rest	75	- 1	1	
	Exercise	89	± 0	11	
	After exercise	76	2	3	
Systemic vascular resistance arb units	Rest	27	- 2	- 3*	
	Exercise	16	- 1	- 3***	**
	After exercise	23	- 3	- 3	
Forearm blood flow ml/100 ml/min	Rest	2.8	0.1	0.4	
	Exercise	2.8	- 0.2	0.9	
	After exercise	3.6	± 0	0.6	
Forearm vascular resistance arb units	Rest	60	-14	-24	
	Exercise	65	14	-30*	
	After exercise	45	-10	-16	
Venous tone mm Hg/ml × 100 ml	Rest	3.0	- 1.0*	- 0.7	
	Exercise	2.9	- 0.9*	- 1.1	
	After exercise	3.3	- 1.5	- 1.8	

Table 1 Central hemodynamics and peripheral circulatory data at rest, during and 4 min after exercise before (control) and after 2 months and 16 months on pindolol. The levels of significance of the changes after 2 months and 16 months compared in the control examination and the difference between the situation after 16 months compared to 2 months at illustrated as * and ** ($p<0.05$, $p<0.01$ and $p<0.001$ respectively).

bo treatment and somewhat, although not significantly, lower than those recorded after 2 months of treatment. Also the pressures recorded during and after exercise were significantly ($p<0.001$) lower than in the control study. Heart rate was lower most strikingly during exercise ($p<0.01$). Cardiac output was slightly lower but the difference from the control was not significant. Stroke volume was significantly higher during exercise ($p<0.05$). The most striking circulatory effect after 16 months of treatment was the decrease in systemic and fore

arm vascular resistance before, during and after exercise. Venous tone was still lower than in the control study.

Differences between 2 and 16 months of treatment
The systolic diastolic and mean arterial blood pressure were numerically lower after 16 months of pindolol treatment than after 2 months but the values obtained were not significantly lower. Heart rate was unchanged. Cardiac output and stroke volume were somewhat higher. The differences,

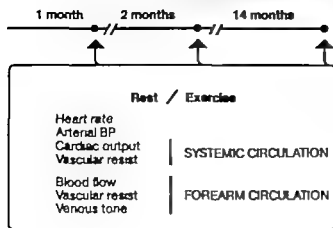


Fig 1 Variables studied.

tion at rest and during exercise. After this examination, treatment with pindolol was started at a dose of 10 mg twice daily. The blood pressure was checked after 1 and 3 weeks of treatment, if the diastolic pressure had not fallen at least 20 mm Hg or if the blood pressure exceeded 160/95 the dose was increased to 15 mg twice daily and again after a further 3 weeks to 20 mg twice daily if the blood pressure had not fallen as indicated above. In this way the desired decrease in blood pressure was achieved in one patient with 20 mg in four patients with 30 mg and in five patients with 40 mg of pindolol daily. The haemodynamic examination was then repeated after a mean duration of treatment of 8 weeks (range 6–11) and after a further 60 (range 52–73) weeks period of treatment.

The haemodynamic examinations were performed in the morning. The patient had taken his ordinary pindolol dosage 2 hours before examination but was otherwise fasting. The analyses were made with the patient in the supine position at rest as well as during and 4 min after an exercise on a bicycle ergometer. The variables studied and the procedure are shown in Figures 1 and 2. For further details see ref (1).

Table 1 summarizes the results obtained after 2 months of pindolol treatment (1) and also includes results after 16 months of treatment (full data to be published in *Acta med Scand.*, 1977). Statistical significance of differences was evaluated by t-tests on paired observations.

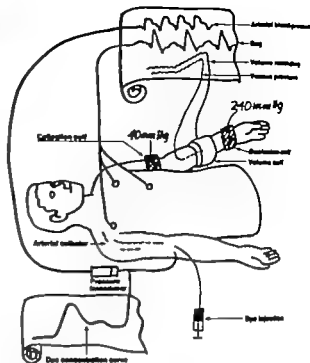


Fig 2 Schematic illustration of the procedure used in the haemodynamic examination.

RESULTS

Effect of 2 months of treatment

The decrease in systolic, diastolic and mean arterial blood pressure was highly significant ($p < 0.001$) both at rest and during exercise. Heart rate was significantly lower at rest ($p < 0.01$), during exercise ($p < 0.01$) and after exercise ($p < 0.05$) as compared to the control situation during placebo treatment. Cardiac output was significantly decreased which was most accentuated during exercise ($p < 0.01$), while stroke volume was unchanged. No effect was noticed on calculated systemic vascular resistance at rest or during exercise, while a slight decrease ($p < 0.05$) was found after exercise. Peripheral resistance in the forearm decreased significantly during exercise ($p < 0.05$). Venous tone was decreased before ($p < 0.05$), during ($p < 0.01$) and after ($p < 0.05$) exercise.

Effect of 16 months of treatment

After 16 months of treatment with pindolol the mean values of the systolic and diastolic arterial blood pressure at rest were 153 ± 4 and 85 ± 12 mm Hg respectively. These values are significantly ($p < 0.001$) lower than those recorded during placebo

		Control	2 months	16 months	Diff 2-16 months
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Stroke volume ml	Rest	75	-1	1	
	Exercise	89	± 0	11	
	After exercise	76	2	3	
Systemic vascular resistance arb units	Rest	27	-2	-3	
	Exercise	16	-1	-3**	**
	After exercise	23	-3	-5**	
Forearm blood flow ml/100 ml/min	Rest	2.8	0.1	0.4	
	Exercise	2.8	-0.2	0.9	
	After exercise	3.6	± 0	0.6	
Forearm vascular resistance arb units	Rest	60	-14	-24	
	Exercise	65	-14	-30*	
	After exercise	45	-10	-16	
Venous tone mm Hg/ml x 100 ml	Rest	3.0	-1.0*	-0.7	
	Exercise	2.9	-0.9*	-1.1	
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Table 1 Central hemodynamics and peripheral circulatory data at rest during and 4 min after exercise before (control) and after 2 months and 16 months on pindolol. The levels of significance of the changes after 2 months and 16 months compared to the control examination and the difference between the situation after 16 months compared to 2 months are illustrated as *^o and ** (p<0.05 p<0.01 and p<0.001 respectively).

to treatment and somewhat, although not significantly lower than those recorded after 2 months of treatment. Also the pressures recorded during and after exercise were significantly (p<0.001) lower than in the control study. Heart rate was lower most strikingly during exercise (p<0.01). Cardiac output was slightly lower but the difference from the control was not significant. Stroke volume was significantly higher during exercise (p<0.05). The most striking circulatory effect after 16 months of treatment was the decrease in systemic and fore-

arm vascular resistance before, during and after exercise. Venous tone was still lower than in the control study.

Differences between 2 and 16 months of treatment

The systolic, diastolic and mean arterial blood pressure were numerically lower after 16 months of pindolol treatment than after 2 months but the values obtained were not significantly lower. Heart rate was unchanged. Cardiac output and stroke volume were somewhat higher the differences,

however being significant ($p < 0.05$) only during exercise. Systemic and forearm vascular resistance were lower and forearm blood flow higher after 16 months of treatment, the difference being significant ($p < 0.05$) during exercise. No difference was found in venous tone.

DISCUSSION

When studying the effect of hypotensive drugs it is important to prolong the observation time and make repeated analyses of the haemodynamic changes to compare with the control situation. This is obvious from the present results which show that although the hypotensive effect of the drug was maintained over a long period of time (in the study 16 months) the mechanisms behind the hypotensive effect changed during the period of treatment. The level of blood pressure is roughly speaking a function of cardiac output and peripheral vascular resistance. Early after the onset of treatment the hypotensive effect was entirely due to a negative chronotropic effect on the heart with unchanged stroke volume lowering the cardiac output with only a small decrease of or no effect on the peripheral resistance. Later on the pressure lowering mechanism was changed to a dominant effect on the periphery while cardiac output was almost normalized, the arterial blood pressure being kept at an unchanged low level. It is tempting to suggest that this gradual decrease in peripheral resistance is due to a partial reversal of structural changes in the resistance vessels as discussed earlier at this symposium. This suggestion is in conformity with findings in spontaneously hypertensive rats that betablockers are able to reduce to some extent already established structural changes (15).

However the picture is complicated by the fact that not all reports on long term studies on the effect of betablockers are in conformity with our findings. Thus Lund-Johansen found that prolonged treatment with alprenolol (5) timolol (6) and atenolol (7) did not decrease peripheral resistance. Similarly Trap-Jensen (1976) recently reported that the hypotensive effect of propranolol was still after one year entirely due to a decrease in cardiac output while peripheral resistance was unchanged. On the other hand Koch (4) found that labetalol (AH 5158) a combined alpha and beta-adrenoceptor blocking agent gave a de-

creased peripheral resistance which conforms with our results on pindolol. These different results are probably due to the fact that the studies have been performed with betablocking agents having different degree of specificity intrinsic activity anaesthetic activity etc. and stress the importance of a careful haemodynamic analysis of each individual preparation of this kind.

The majority of studies on the antihypertensive effects of betablocking agents deal merely with the situation at rest. However analysis of the haemodynamic events during and after physical exercise add valuable information on the effect of the antihypertensive treatment. It is important to determine whether the hypotensive effect obtained at rest is maintained during physical work similar to that in which the patient is engaged in daily life. As is evident from the present study the effect of pindolol after two months of treatment was even more pronounced during exercise than at rest for some important variables such as heart rate, cardiac output, forearm vascular resistance and venous tone. The same was true after 16 months of treatment.

Venous tone of the deep forearm was found to be significantly lower during pindolol administration as compared to the control situation. Earlier Walsh et al. (14) have found reduced venous distensibility in hypertensive patients, which was normalized by various antihypertensive drugs such as chlorothiazides, reserpine and methyldopa. Studies in animals confirm these findings. Thus Overbeck (9) observed a shift of the venous pressure-volume relation towards the pressure axis in experimentally induced renal hypertension in the dog. Similarly Simon (11) recently found a decreased venous capacity in the spontaneously hypertensive rat. If this reduction in venous compliance is a result of a neurogenic vascular influence or an increased water content of the venous wall is not yet definitely settled. If however the increased venous tone in hypertensive patients is due to an increased venomotor activity as postulated by Walsh et al. (14) this is probably mediated by alpha-receptors (3). Since no basis for an alpha-adrenergic blocking action of pindolol has hitherto been demonstrated the mechanism by which pindolol decreased venous tone in the present cases of essential hypertension is not clear. The effect might be due either to the intrinsic

activity of pindolol or an effect on the central nervous system.

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DISCUSSION

Olav Thulesius

Increased vascular reactivity to vasoconstrictor stimuli has been considered to be an important mechanism in the pathogenesis of essential hypertension. This is believed to be connected with an increased sensitivity of the vascular smooth muscle cells to physiological stimulation, e.g. with the neurotransmitter noradrenaline. Another possible explanation may be altered geometrical proportions of the vascular wall, with an increased wall/lumen ratio, a mechanism, which in itself for purely anatomical reasons, gives rise to an altered vascular response pattern with increased vasoconstriction, with normal adrenoceptor stimulation. In the Department of Clinical Physiology and Surgery of V r s Central Hospital, Jan-Erik G rre, Erik Berlie and I undertook a study to test these hypotheses.)

Arterial biopsies were obtained in abdominal surgery on 11 hypertensive and 21 normotensive subjects. The hypertensive subjects had mild to moderate essential hypertension and only 7 of them were being treated with antihypertensive drugs. Treatment was interrupted prior to the operations.

The arterial biopsies were immediately put into chilled Krebs solution and then cut into helical strips. Thereafter they were suspended between a clamp and transducer permitting the recording of isometric tension. The organ bath was continuously aerated with 95% oxygen and 5% carbon dioxide and temperature was kept at 37 C.

Each strip was equilibrated for two hours. Noradrenaline was then administered.

Values of threshold and maximal contraction are plotted as a function of blood pressure in Figures 1 and 2. From these it can be seen that the threshold values for noradrenaline are almost identical for all the subjects tested. The same holds for ED₅₀ and the force of maximal concentration. A regression analysis of systolic blood pressure versus maximal tension showed slight correlation of maximal tension to increasing arterial blood pressure. It is possible that this correlation might be more pronounced if further patients with higher pressure could be included in the material.

The present results do not lend support to the assumption that arterial strips from patients with essential hypertension are more responsive to the

) This study was supported by grants from Tore Nilsson f nd

however being significant ($p < 0.05$) only during exercise. Systemic and forearm vascular resistance were lower and forearm blood flow higher after 16 months of treatment, the difference being significant ($p < 0.05$) during exercise. No difference was found in venous tone.

DISCUSSION

When studying the effect of hypotensive drugs it is important to prolong the observation time and make repeated analyses of the haemodynamic changes to compare with the control situation. This is obvious from the present results which show that although the hypotensive effect of the drug was maintained over a long period of time (in the study 16 months) the mechanisms behind the hypotensive effect changed during the period of treatment. The level of blood pressure is roughly speaking a function of cardiac output and peripheral vascular resistance. Early after the onset of treatment the hypotensive effect was entirely due to a negative chronotropic effect on the heart with unchanged stroke volume lowering the cardiac output with only a small decrease of or no effect on the peripheral resistance. Later on the pressure lowering mechanism was changed to a dominant effect on the periphery while cardiac output was almost normalized, the arterial blood pressure being kept at an unchanged low level. It is tempting to suggest that this gradual decrease in peripheral resistance is due to a partial reversal of structural changes in the resistance vessels as discussed earlier at this symposium. This suggestion is in conformity with findings in spontaneously hypertensive rats that betablockers are able to reduce to some extent already established structural changes (15).

However the picture is complicated by the fact that not all reports on long term studies on the effect of betablockers are in conformity with our findings. Thus Lund-Johansen found that prolonged treatment with alprenolol (5), timolol (6) and atenolol (7) did not decrease peripheral resistance. Similarly Trap-Jensen (1976) recently reported that the hypotensive effect of propranolol was still after one year entirely due to a decrease in cardiac output while peripheral resistance was unchanged. On the other hand Koch (4) found that labetalol (AH 5158) a combined alpha and beta-adrenoceptor blocking agent gave a de-

creased peripheral resistance which conforms with our results on pindolol. These different results probably due to the fact that the studies have been performed with betablocking agents having different degree of specificity, intrinsic activity, anaesthetic activity etc. and stress the importance of a careful haemodynamic analysis of each individual preparation of this kind.

The majority of studies on the antihypertensive effects of betablocking agents deal merely with the situation at rest. However analysis of the haemodynamic events during and after physical exercise add valuable information on the effect of the antihypertensive treatment. It is important to determine whether the hypotensive effect obtained at rest is maintained during physical work similar to that in which the patient is engaged in daily life. As is evident from the present study the effect of pindolol after two months of treatment was even more pronounced during exercise than at rest for some important variables such as heart rate, cardiac output, forearm vascular resistance and venous tone. The same was true after 16 months of treatment.

Venous tone of the deep forearm was found to be significantly lower during pindolol administration as compared to the control situation. Early Walsh et al. (14) have found reduced venous distensibility in hypertensive patients, which was normalized by various antihypertensive drugs such as chlorothiazides, reserpine and methyldopa. Studies in animals confirm these findings. Thus Overbeck (9) observed a shift of the venous pressure-volume relation towards the pressure axis in experimentally induced renal hypertension in the dog. Similarly Simon (11) recently found a decreased venous capacity in the spontaneously hypertensive rat. If this reduction in venous compliance is a result of a neurogenic vascular influence or an increased water content of the venous wall is not yet definitely settled. If however the increased venous tone in hypertensive patients is due to an increased venomotor activity as postulated by Walsh et al. (14), this is probably mediated by alpha-receptors (3). Since no basis for an alpha-adrenergic blocking action of pindolol has hitherto been demonstrated the mechanism by which pindolol decreased venous tone in the present cases of essential hypertension is not clear. The effect might be due either to the intrinsic

was carried out on patients with coronary insufficiency.

Eight men age 43–57 with severe angina pectoris, pathological ECG-reactions in work tests and low maximal working capacity were catheterized. Arterial blood pressure and heart frequency were registered in the supine position at rest and during work and cardiac output was determined according to Fick's principle. After 30 minutes rest, pindolol 0.014 mg/kg body weight was given intravenously and the examination procedure was repeated after a further 30 minutes.

The mean systolic blood pressure decreased during work ($p < 0.05$) as a result of a decreased cardiac output ($p < 0.05$) while the peripheral vascular resistance was unchanged (Figure 1). The decrease of the cardiac output was mainly due to a lowered heart rate ($p < 0.01$) while stroke volume increased ($p < 0.01$) (Figure 2).

In conclusion, pindolol had an effect, in this trial, which was more like that of selective beta-blockers than non-selective ones.

Torbjörn Lundman

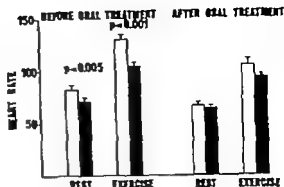
We have measured the acute effect of propranolol before and after long-term oral treatment, in a trial performed in the Serafiner Hospital with Brundin and Edhag (Br Heart J Vol 38 1065–1072, 1976).

Eight patients with moderate hypertension were studied in hospital, first haemodynamic study was done before treatment. The patients were then given propranolol and after long-term treatment (2–9 months), they were taken into hospital again and propranolol was withdrawn for three days. That time is enough for the drug to disappear from the blood and also for the metabolites to disappear from the blood and from the heart tissue.

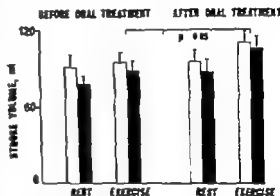
The haemodynamic measurements were done first at rest, then during exercise. Following a short pause new basal measurements were performed. Then propranolol was infused in a dose of 0.2 mg/kg body weight. After five minutes new measurements were performed at rest and during exercise. Pressures were recorded in the right heart, in the subclavian artery and the cardiac output was measured with the Fick principle.

Figure 1 shows the results for heart rate. There was a significant reduction of heart rate after intravenous propranolol both during rest and during exercise. But after the long-term treatment, propranolol injection did not significantly reduce heart rate either at rest or during exercise.

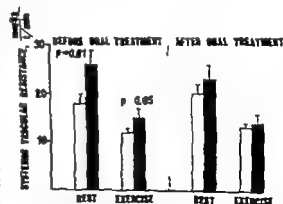
Figure 2 shows the results for stroke volume. Propranolol injection caused no significant changes either before or after the long-term oral therapy. However the spontaneous stroke volume during exercise was significantly increased after long-term treatment in a way similar to that seen after a period of intense physical training which is in agreement with the results found by Pernow et al presented here today.



Lundman Fig. 1



Lundman Fig. 2



Lundman Fig. 3

Figure 3 shows the results for systemic vascular resistance. Before long-term treatment the systemic vascular resistance was significantly increased both at rest and during exercise. After long-term treatment no significant effects were obtained by propranolol injection.

To conclude the study shows that haemodyna-

sympathetic neurotransmitter noradrenaline. Maximal tension was slightly higher in strips from hypertensive patients, but the difference was not statistically significant ($p < 0.10$)

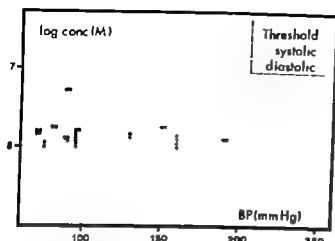


Fig 1 Threshold towards noradrenaline (M =molar) in relation to systolic and diastolic blood pressure

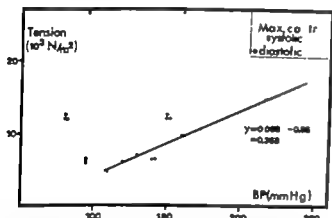


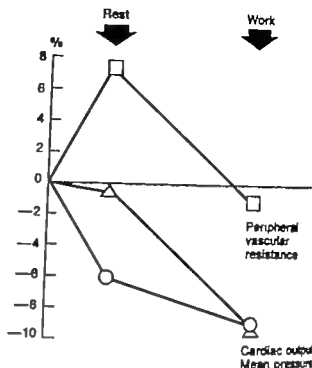
Fig 2. Maximal force to contraction given as tension (N =Newton) per cross-sectional area (m^2) in relation to systolic and diastolic blood pressure. Regression equation correlates systolic pressure and tension

Per Bjerle

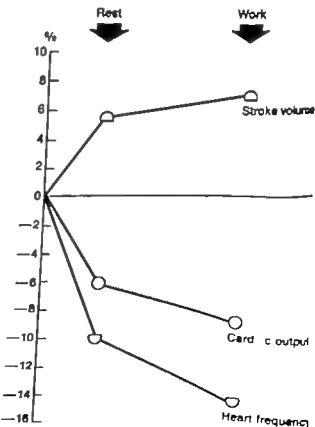
In many studies it has been shown that the anti-hypertensive effect of beta blockers develops gradually.

Initially most beta-blockers raise peripheral vascular resistance, but then during continued therapy the resistance gradually returns to the initial value.

Professor Pernow has now reported a statistically significant decrease of peripheral vascular resistance after 8 weeks of pindolol treatment. But what happens initially? To answer that question the following study carried out at the departments of Cardiology and Clinical Physiology at the university of Umeå may be of interest although it



Bjerle Fig. 1



Bjerle Fig. 2.

Clinical Pharmacology of Beta Adrenoceptor Blocking Agents

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Clinical Pharmacology Laboratory Lung Clinic Renströmska Hospital Göteborg Sweden

mic changes remain more than 72 hours after withdrawal of long-term treatment with propranolol in the form of reduced heart rate both at rest and during exercise, increased stroke volume during exercise and lack of circulatory response to acute propranolol administration.

Ramon Sivertsson

Pernow's paper showing that pindolol reduces resistance, was very interesting. With this pindolol effect in mind, I would like to comment further on the results, which I showed you before. After 6 months treatment with atenolol we observed a considerable rise in resistance. Treatment with mefruside, the saluretic drug, did not change the resistance. My question to the audience is now: What conclusions can be drawn from these haemodynamic findings regarding the practical use of these drugs? Should we prefer one drug rather than another and if so which drug?

One more comment, and this is to Pernow's discussion. It is a very interesting thought that structural changes may be responsible for the change in the haemodynamic picture between 2 and 16 months. But I do not think, that the results presented can prove such a change. The forearm resistance values did not refer to maximal dilatation, as far as I understood, but rather to a resting situation in the arm. If so changes in smooth muscle tone can explain the results as well.

Per Lund Johansen

Just a short comment on the question about the possibility of reversing structural changes. In one of the last issues of "Circulation" there is a very interesting paper on patients who have been operated on for coarctation in the aorta and been restudied ten years later. The blood flow and the resistance to flow were measured in the arms and in the legs. In spite of the fact that the operation was successful, pathological changes in blood flow and resistance in the arms were still demonstrable. In the legs and the vascular areas which had been protected from the high blood pressure blood flow and resistance were still normal.

Göran Berglund

I have a question for Ramon Sivertsson as well. Did you find any correlation between the blood pressure decrease and the decrease in resistance at maximal dilatation? In other words, is a good blood pressure decrease characterising those patients who respond with a decrease in resistance at maximal dilatation?

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The last study I mentioned is not finished but as far as I can remember there is a correlation between change in pressure and change in resistance at rest.

Olov Thulesius

I have a question for Bengt Pernow: I think it is very important to look at changes in venous tone and these have not been studied as much as they should be, but I think that such a study involves a very difficult technique. I noticed that you had an increase in venous pressure but no increase in volume. You measured changes during an increase in both volume and pressure, but you do not know the basal blood volume in the vessels. In order to compare sets of measurements, it is essential to know the initial volume in the vessel, and that is very hard to determine.

Bengt Pernow

I agree with Thulesius, that this is a very tricky technique and perhaps that is why so few studies have been performed on the compliance vessel situation of patients. As I showed, this technique by Sharpey Schafer records the increase in volume and the increase in pressure simultaneously and from this ratio one can get a rough idea of the venous tone. There are other methods, which we have also used. One can get a pressure volume curve by increasing the venous pressure of the forearm setting the pressure at different levels. One gets almost the same results with this technique.

Kjell Haglund

It is often stated that beta-adrenergic blocking drugs have no acute antihypertensive effects. We have found with metoprolol, which is a cardio-selective beta-blocking agent and probably also with alprenolol that after peroral medication the blood pressure decreased significantly within one hour and was still significantly decreased eight hours after administration. We do not think, that you will have to wait a fortnight or more to get the antihypertensive effect.

Hans Dunér

It is difficult to summarize this part of the symposium on haemodynamics in essential hypertension. The subject is complex which was apparent not least from the excellent survey we heard from Lennart Hansson.

However it is striking how well the haemodynamic pattern with successive reduction of cardiac output and increase of peripheral resistance during the development of hypertension as described by Per Lund Johansen fits with the concept derived from the results of Ramon Sivertsson's study on the appearance of structural changes in the resistance vessels. His conclusion that these structural vascular changes might be partially reversible during long-term blood pressure lowering therapy is in agreement with the results which Bengt Pernow reported today.

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Clinical Pharmacology of Beta Adrenoceptor Blocking Agents

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Pindolol A Pharmacokinetic Comparison with other Beta Adrenoceptor Blocking Agents

Jürg Meier

*From the Biopharmaceutical Department Sandoz AG
Basle Switzerland*

Abstract. The pharmacokinetic properties of the nine most frequently studied β -adrenoceptor blocking agents (alprenolol, atenolol, metoprolol, oxprenolol, pindolol, practolol, propranolol, sotalol and timolol) were compared. Some pharmacokinetic parameters were found to be quite similar or at least not relevantly different in this group of drugs: e.g. the rate and the amount of absorption, the distribution volume, the elimination half-life, the total clearance and the total urinary excretion. However some other pharmacokinetic parameters: e.g. the daily dosage, the oral bioavailability (the first-pass effect and the ratio of renal versus hepatic clearance, were surprisingly different in this series of drugs. A good correlation between systemic availability and urinary excretion of unchanged drug could be demonstrated for the beta-adrenoceptor blocking agents.

The pharmacokinetic comparison in this series of beta-blockers revealed that pindolol has some relevant advantages. Due to the moderate metabolism, negligible first-pass effect results which yield a high oral bioavailability and leads to small interindividual variations in plasma levels and of facts. As a result of the balanced clearances by the liver and the kidney and probably some compensating factors, no accumulation of pindolol has to be expected in patients with partial liver or kidney impairment. Therefore, apart from possessing the lowest daily dosage of the studied beta-blockers, pindolol administration is relatively less likely to be critical in patients with partial renal or hepatic insufficiency.

The clinical pharmacokinetics of most β -adrenoceptor blocking drugs have been well studied in the last few years. The most recent and comprehensive review has been published by Johnson

and Regårdh (12). In this paper the nine most frequently studied beta-adrenoceptor blocking drugs are compared in respect to their pharmacokinetic similarities and differences. Some newer data of pindolol from various authors will be included and the clinical significance of the observed pharmacokinetic differences will be discussed.

SIMILARITIES AND DIFFERENCES

In view of the chemical structures of the beta-adrenoceptor blocking drugs, both similarities and differences can be expected in the pharmacokinetic parameters. The isopropylaminopropoxy side chain is common to seven of the nine β -blockers listed in Table 1. Sotalol and timolol have slightly different side chains. The bulk of the molecules consists of an aromatic ring system, however which is different for each beta-adrenoceptor blocking agent (12).

The pharmacokinetic parameters of β -blockers which are similar or at least not in a clinically significant way different, are summarized in Table 1. All the beta-adrenoceptor blocking agents compared have a fast and complete absorption. One possible exception is atenolol (9,14) for which a slower and apparently less complete absorption is reported. In all cases the distribution volumes exceed by far the available body space. Therefore the beta-adrenoceptor blocking agents are well distributed into the tissues. The plasma protein binding is generally low for the beta-blockers. The protein binding is highest for propranolol (93%), and alprenolol (85%); but even in these cases the plasma protein binding is not critical in view of the large distribution volume. No drug interactions based on a displacement mechanism are known or to be expected.

Table 2. Pharmacokinetic parameters of β -blockers which are different.

β -blocker	Daily dose mg	Bioavailability oral %	Dose dependent bioavailability	Urinary excretion unchanged drug %	First-pass effect %	Accumulation expected	
						Liver disease	Renal failure
Alprenolol	400	≈ 10	Yes	<1	≈ 90	—	—
Almolol	200	≥ 40	No	≈ 40	<15	—	—
Metoprolol	300	≈ 50	No	≈ 3	≈ 50	—	—
Oxprenolol	160	24–60	No	2–5	30–30	—	—
Pindolol	15	87	No	≈ 40	13	No	No
Practolol	400	100	No	>90	0	No	Yes
Propranolol	300	30	Yes	<1	≈ 60	Yes	Yes
Sotalolol	240	≥ 60	Not likely	≈ 60	≤ 15	—	Yes
Theolol	30	—	Not likely	≈ 20	—	—	—

[Partly from Johnson and Regårdh, *Clinical pharmacokinetics* 1 233 1976]

the first-pass effect. The disadvantage of a strong first-pass effect is not only the lower availability after oral administration, which can be compensated by a higher dosage, but also the resulting larger biological, individual variation in the plasma levels and drug response (2,22). For alprenolol and propranolol the bearing of the first-pass effect on the pharmacokinetics is accentuated by the resulting non-linear oral bioavailability. On the other hand, the formation of pharmacologically active metabolites, as reported by Ablad et al. (1) Bodin et al. (5) and Paterson et al. (20) for these two drugs, can partly compensate the disadvantages of the strong first-pass effect. However the active metabolite 4-hydroxy-propranolol contributes only with a smaller and even faster eliminating plasma level (20) than the parent drug itself.

For pindolol a negligible first-pass effect is re-

ported in man (15). An investigation of the absorption and the first-pass effect of pindolol in the dog (16) revealed that the low first-pass effect observed with pindolol in man is not a property of the molecule as such, but rather correlates with the extent of metabolism. This is demonstrated in Figure 1 where the systemic availabilities of a few beta-adrenoceptor blocking agents are plotted against their urinary excretion on a logarithmic scale. This representation is of course analogous to a graph in which the magnitude of the first-pass effect is plotted against the extent of metabolism. According to Figure 1 practolol is not metabolised and has a very good systemic availability. In the pindolol is eliminated to about 40% as unchanged drug in the urine and has an almost complete oral bioavailability (87%) with a negligible first-pass effect (15). Next in the row is pindolol in the dog with a rather strong hepatic degradation and a medium first-pass effect (16). Then metoprolol, oxprenolol, propranolol and finally alprenolol, which is almost completely metabolised and has a very strong first-pass effect, are following in Figure 1. As expected from the different first-pass effects of beta-blockers, the individual variations are large for e.g. alprenolol (2,22) and propranolol (8) and small for e.g. pindolol after both single and multiple oral administration (10). Since there is a definite correlation between plasma levels and effects (21) these differences are of clinical relevance, even though the plasma level response curve seems rather flat (21).

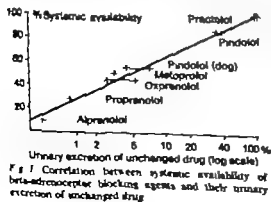


Fig. 1 Correlation between systemic availability of beta-adrenoceptor blocking agents and their urinary excretion of unchanged drug.

Table 1 Pharmacokinetic parameters of β -blockers which are similar

β -blocker	Absorption (H) % of dose T_{max}		Distribution volume l/kg	Protein binding (human serum) %	Elimination half-life (H)	Total clearance (l/min)	Total urinary excretion (%)
Alprenolol	0.5-1.5	90	3.3	85	2-3	1.2	>90
Atenolol	2-4	>50	—	—	6-9	—	—
Metoprolol	0.5-1.5	>95	5.6	12	3-4	1.1	>95
Oxprenolol	0.5-1	70-95	1.2	—	1-2	0.6	70-95
Pindolol	1.5-2	>95	2.0	40	3-4	0.4	>90
Practolol	1-3	>95	1.6	—	5-13	0.14	>90
Propranolol	1-3	>90	3.6	93	2-3	1.0	>90
Sotalol	2-3	>75	—	—	5-6	—	—
Timolol	1-3	>90	—	—	4-5	—	75

[Partly from *Johnsson and Regårdh, Clinical pharmacokinetics*, 1, 233 1976]

In general the elimination half lives of the beta blockers are short and range between 1-2 hours for oxprenolol up to 5-13 hours for practolol. Most beta-blockers are therefore dosed three times daily. However, twice or once daily application is possible for beta-blockers, because the effects do not decline linearly with the plasma levels but with a slower zero-order kinetics (13). The response of beta blockers is therefore longer than we might expect from the relatively short elimination half lives. The clearance of practolol is lowest due to the slowest elimination constant. The beta-blockers and/or their metabolites are mainly cleared via the kidney (70-100% of the absorbed dose). This column in Table 1 refers to the total urinary excretion of radioactively labelled drug substance which includes the parent drug and its metabolites.

Table 2 summarizes the pharmacokinetic parameters of beta-adrenoceptor blocking drugs which are different and have a clinical relevance in their difference. The daily dosage varies greatly from pindolol (about 15 mg per day) to alprenolol and practolol (about 400 mg per day). Based on "ecological" considerations (clearance, saturation of enzyme and eliminationsystems, drug interactions and competitive bindings) a small amount of a potent drug is advantageous for a patient especially in chronic treatment and when many other drugs have to be administered simultaneously. Large differences can further be observed in the oral bioavailabilities which are highest for practolol and pindolol (100 and 87%) and lowest for al-

prenolol and propranolol (10 and 30%). There is no direct correlation between bioavailability and necessary daily dosage because the two highest dosed beta-blockers, alprenolol and practolol, have the lowest and highest bioavailability. In two cases, alprenolol and propranolol, the strong first-pass effect which is responsible for the low oral bioavailability is not dose linear due to the saturation of drug extraction by the liver (12). Great differences are also observed in the column "urinary excretion of unchanged drug". Practolol is practically not metabolised but excreted as unchanged drug by the kidneys. On the other hand, alprenolol, metoprolol, oxprenolol and propranolol are very intensively metabolised and very little unchanged drug is excreted in the urine. Atenolol, pindolol, sotalol and timolol are cleared to various degrees by the kidneys and by the liver. The main differences in the pharmacokinetics of beta-adrenoceptor blocking agents are therefore the "first-pass effect" and "the clearance". These topics will be elaborated in the following paragraphs.

FIRST PASS EFFECT

Great variations in the oral bioavailabilities of the nine beta-adrenoceptor blocking agents are listed in Table 2, even though the absorption has been shown to be fast and complete in Table 1. The major cause of the low bioavailability of most beta-adrenoceptor blocking agents is a high first-pass effect. Metabolism by the liver during its first passage after oral absorption is referred to as

Table 3 Pharmacokinetically based advantages of Pindolol in therapy

PHARMACOKINETIC FACTS

- High bioavailability
- Low first-pass effect
- Moderate metabolism
- Low protein binding
- Balanced clearance by the kidney and the liver
- Partly compensating factors in liver or kidney insufficiency

THERAPEUTICAL ADVANTAGES

- Low daily dosage
- No saturation effects
- Small variations in plasma levels and effects
- No accumulation of Pindolol in patients with renal or hepatic impairment (not critical in dosing)

low first-pass effect and the low dosage: small variations in plasma levels and effects due to the low first-pass effect, the moderate metabolism and the low protein binding; and finally no accumulation of pindolol has to be expected in patients with renal or hepatic impairment due to the balanced clearance and probably some compensating factors

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CLEARANCE OF β -ADRENOCEPTOR BLOCKING AGENTS

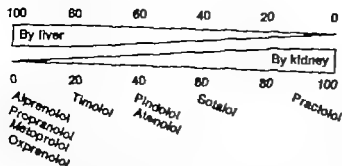


Fig 2 Clearance of beta-adrenoceptor blocking agents by the liver and/or the kidney

CLEARANCE

Figure 2 demonstrates the clearance of the nine beta-adrenoceptor blocking agents by the kidney and/or the liver. There is a large difference in the way of clearance. Practolol is almost completely cleared by the kidneys. In kidney insufficiency, therefore the dosage has to be reduced due to the smaller elimination kinetics (6, 3). On the other hand the beta-blockers alprenolol, propranolol, metoprolol and oxprenolol are almost completely eliminated by hepatic metabolism. For propranolol Branch et al. (7) could show that the clearance decreased with the evidence of increasing severity in liver function impairment. Pindolol and probably also timolol, atenolol and sotalol are cleared partly by the kidneys and partly by the liver. On theoretical grounds, an impairment of the liver or kidney function cannot be so critical. For pindolol it could be shown experimentally that the dosage regimen is not critical in patients with renal or hepatic impairment and that probably even a certain degree of compensating clearance occurs if the function of the liver or the kidney is reduced. Ohnhaus et al. (17) determined the elimination constants of pindolol in patients with varying degrees of kidney impairment. The elimination constants varied between 0.1 and 0.3 hours⁻¹ without significantly decreasing even at very low or zero creatinine clearance. Oie and Levy (19) found a positive correlation between the renal clearance of pindolol and the creatinine clearance, but no statistically significant correlation ($n=24$ patients) was observed between the important over all clearance of pindolol and the creatinine clearance. In a very recent investigation (11) pindolol showed a decreased clearance in hypertensive patients with

impaired renal function after intravenous and oral administration. However due to a reduced absorption after oral administration, the plasma levels in the group with impaired renal function were only slightly higher (maximal plasma level 40 instead of 30 ng/ml after single dose or simulated multiple dosing) compared to the patients with normal renal function. Therefore, no relevant accumulation of pindolol seems to occur in patients with renal impairment, whereas for propranolol Bianchetti et al. (4) report a 7 to 10-fold increase of plasma concentrations in uraemic patients. Propranolol should be used with great caution and at low doses in chronic renal failure according to these authors (4). For sotalol the mean plasma half-life was prolonged from 5 hours (normal) to 42 hours in patients with renal failure (23). Little is published about the pharmacokinetics of beta-blockers in liver disease. For propranolol (12) the drug clearance decreased in liver disease to 0.29 L/min (normal 1.0 L/min) and the plasma half-life increased to 18 hours (normal 2 to 3 hours). Due to the smaller extent of hepatic extraction the total clearance of pindolol seems to be less affected in patients with liver disease. No correlation has been found between antipyrine clearance (parameter of the metabolic capacity of the liver microsomal enzyme system) and the total body clearance of pindolol since some patients with intact renal function excreted a higher portion of pindolol in the urine as liver function decreased (18). Only in cases with real liver failure (antipyrine clearances less than 10 ml/min) did the clearance of pindolol decrease (18), and the dosage would have to be adjusted.

CONCLUSIONS

There are a number of similarities in the pharmacokinetic properties of the current beta-adrenoceptor blocking agents (Table 1). There are also some different pharmacokinetic parameters (Table 2), some of which are of clinical relevance. Especially for pindolol (Table 3) quite a series of interesting and valuable pharmacokinetic parameters can be summarized (left side of Table 3). The resulting therapeutic advantages are the following: a low daily dosage based on the high bioavailability, the low first pass effect, the moderate metabolism and mainly of course due to the potency of the drug, no saturation effects due to the

Investigations with Beta Adrenoceptor Blocking Drugs in Healthy Volunteers

Walter H. Aellig

*From the Experimental Therapeutics Department Biological & Medical Research Division Sandoz AG
Basle Switzerland*)*

Abstract. Several properties of beta-adrenoceptor blocking drugs can be investigated in healthy volunteers with simple non-invasive techniques giving reproducible results.

Beta-adrenoceptor blocking activity can be evaluated in several ways. One is to compare heart rate during physical exercise before and after drug administration. 5 mg pindolol and 100 mg propranolol were about equiactive in this test.

Another method is to administer i.v. infusions of isoprenaline before and after the administration of the antagonist and to determine the dose of isoprenaline required to increase heart rate to 120 beats/min. Pindolol is about 40 times more potent than propranolol and Sandoz 23-784 about 10 times more potent than pindolol in this test.

The moderate intrinsic sympathomimetic activity (ISA) of pindolol leads to a slight increase in resting heart rate of subjects in the supine position when sympathetic tone is very low. In the sitting position pindolol like propranolol (without ISA) reduces resting heart rate (but to a lesser extent). Sandoz 23-784, a drug with high ISA, increases resting heart rate both in the supine and in the sitting position. For comparative studies on the duration of action of different drugs exercise-induced tachycardia seems most convenient. Experiments have shown that in equipotent doses pindolol (5 mg) exhibits a longer duration of action than propranolol (100 mg).

Studies on the beta-adrenoceptor blocking activity of pindolol after oral and after i.v. administration support the results of pharmacokinetic studies showing nearly complete absorption and a small first pass effect for this drug in man.

**) This paper is 15 thirds of lectures given at this symposium and at the symposium Beta-blockade Hypertension Basle 27/28 January 1977*

INTRODUCTION

The aim of the present symposium is to discuss various aspects of hypertension and especially its treatment. The beneficial effect of beta-adrenoceptor blocking drugs in the treatment of hypertension is well established. Many different beta-adrenoceptor blocking drugs are available today and all are effective in the treatment of hypertension, and, of course, also angina pectoris. The therapeutic effectiveness in these indications is attributed to their clearly defined pharmacological action and not to a nonspecific effect, such as local anaesthetic activity (9, 10, 11).

The spectrum of pharmacological properties of these various drugs is not identical. In addition to their common property of blocking beta-adrenoceptors, some compounds possess e.g. intrinsic sympathomimetic activity. The duration of action of the different substances is not the same and also the bioavailability varies from drug to drug. In my contribution to this symposium I would like to discuss how several properties of beta-adrenoceptor blocking drugs can be investigated in healthy volunteers with simple non-invasive and reproducible techniques. Most of the examples shown are taken from studies with pindolol and propranolol (1, 2, 3, 4).

ACTIVITY

Beta-adrenoceptor blockade can be evaluated in healthy volunteers in several different ways. One is to compare heart rate and systolic blood pressure during physical exercise before and after drug administration. An example of an experiment with pindolol (5 mg p.o.) (3) is shown in Figure 1. Five healthy volunteers exercised on a bicycle ergometer with a work load of 150 Watts for 3 min. This produced a mean increase in heart

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brospinal fluid but the effect of the drug with the central nervous system is unclear

Nils Svedmyr

I think that all beta blockers enter the blood-brain barrier

DISCUSSION

Lennart Hansson

I would like to say a few words to Dr Meier. I think that you perhaps overstate the importance of a high first pass effect. Your criticism is valid only if the metabolites are not active. But in the case for example of alprenolol and propranolol we know that the metabolites are also active. For the clinician it is the degree of beta blockade that is of interest and he does not care whether that is obtained with the initial drug or with its metabolites.

Bengt Pernow

Are there any data on the passage of these drugs through the blood brain barrier?

Pavel Jerie

Pindolol passes into the brain after 10 to 12 minutes and this can be shown by its effect on the EEG

Hans Åberg

Neither atenolol nor metoprolol seem to penetrate the blood brain barrier

Kjell Haglund

I do not agree with Hans Åberg. We know that metoprolol and probably atenolol enter the cere

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INTRODUCTION

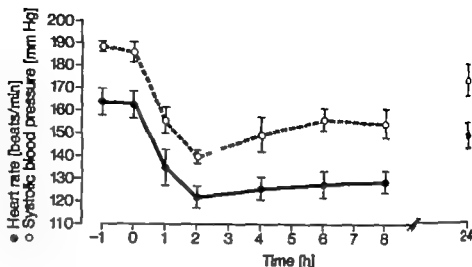
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The spectrum of pharmacological properties of these various drugs is not identical. In addition to their common property of blocking beta-adrenoceptors some compounds possess e.g. intrinsic sympathomimetic activity. The duration of action of the different substances is not the same and also the bioavailability varies from drug to drug. In my contribution to this symposium I would like to discuss how several properties of beta-adrenoceptor blocking drugs can be investigated in healthy volunteers with simple non-invasive and reproducible techniques. Most of the examples shown are taken from studies with pindolol and propranolol (1-4, 3-4).

ACTIVITY

Beta-adrenoceptor blockade can be evaluated in healthy volunteers in several different ways. One is to compare heart rate and systolic blood pressure during physical exercise before and after drug administration. An example of an experiment with pindolol (5 mg p.o.) (3) is shown in Figure 1. Five healthy volunteers exercised on a bicycle ergometer with a work load of 150 Watts for 3 min. This produced a mean increase in heart

Fig 1 Heart rate and systolic blood pressure at the end of 3 min exercise (150 Watt) on the bicycle ergometer before and after oral administration of 5 mg pindolol (mean \pm s.e. $n=5$ data from Aellig, 1976 [3])



rate from 96 ± 6 (mean \pm s.e.) to 163 ± 6 beats/min. Systolic blood pressure was increased from 121 ± 3 mm Hg to 186 ± 5 mm Hg.

The figure shows that heart rate at the end of exercise was markedly reduced within one hour after oral administration of pindolol and reached its minimum with 122 ± 5 beats/min after 2 hours. Systolic blood pressure at the end of exercise was reduced in a similar way to a value of 140 ± 3 mm Hg after 2 hours. If we carry out the same experiment with different drugs, the maximum reduction in exercise induced tachycardia is a measure of the relative activity of the different doses of the drugs investigated. In the example shown in the figure we also studied the effect of propranolol and it was found that the maximum reduction of exercise induced tachycardia was also observed 2 hours after oral administration. The effect of 100 mg propranolol was with 41 beats/min comparable to that seen after 5 mg pindolol (42 beats/min). As higher doses of both drugs produced a greater reduction in exercise induced tachycardia, it was concluded that the effects seen after the doses used of both drugs were still on the ascending part of the dose-response curve and that therefore 5 mg of pindolol and 100 mg propranolol were about equiactive in this test.

Another way to assess comparative beta-adrenoceptor blocking activity is to administer intravenous infusions of the beta-adrenoceptor stimulant drug isoprenaline before and after administration of a beta-adrenoceptor blocking drug and to determine the dose of isoprenaline which is re-

quired to produce a given increase in heart rate or a given heart rate value. Although intravenous infusions of isoprenaline are easy to administer and produce reproducible results, exercise-induced tachycardia seems to be more closely related to the physiological beta adrenoceptor stimulation occurring in normal life.

In our experiments isoprenaline hydrochloride was infused into a left forearm vein of the subject resting in the supine position. The starting dose was $2 \mu\text{g}/\text{min}$ and was doubled every 5 minutes until a heart rate of at least 120 beats/min was reached. A higher starting dose was used after the administration of the beta-adrenoceptor blocking drugs.

Increasing doses of the beta adrenoceptor blocking drugs lead to a dose-dependent parallel shift of the isoprenaline dose-response curves to the right. This is typical for a competitive antagonism. For any dose of a beta-adrenoceptor blocking drug a dose of isoprenaline can be found which will produce a given increase in heart rate; there exists no "complete beta-blockade". From these curves the dose of isoprenaline required to increase heart rate to 120 beats/min was determined for every subject after each dose of the different drugs.

Figure 2 shows the results of a comparative study with pindolol and propranolol (3) and of a recent investigation with Sandoz 23-784 (4-(3-tert. Butylamino-2-pivaloyloxypropoxy)-2-indolinone hydrochloride). It is evident that all three beta adrenoceptor blocking drugs lead to a dose-dependent increase of the dose of isoprenaline re-

Fig. 2. Dose of isoprenaline required for a heart rate of 120 beats/min after oral administration of various doses of pindolol ($n=3$), propranolol ($n=3$), and 23-784 ($n=4$) (data for pindolol and propranolol from Aeling, 1976 [3]).

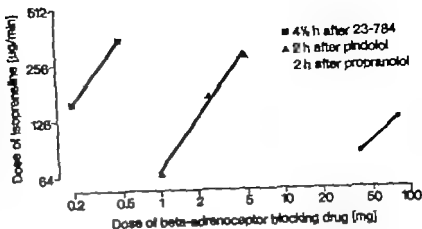
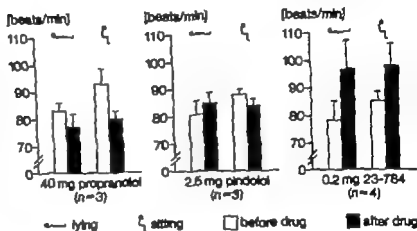


Fig. 3. Resting heart rate in the lying and in the sitting position before and two hours after oral administration of 2.5 mg pindolol and 40 mg propranolol and before and 4 1/2 hours after 0.2 mg 23-784 (mean \pm s.e., data for pindolol and propranolol from Aeling, 1976, [3,5]).



quired to achieve a heart rate of 120 beats/min. The times chosen for the determination of the isoprenaline dose (i.e. 2 hours after pindolol and propranolol and 4 1/2 hours after 23-784) were those at which maximum effect was seen. The dose-response curves in Figure 2 are practically parallel and as evaluation of the distances between them shows that pindolol is about 40 times more potent than propranolol (3) and 23-784 is about 10 times more potent than pindolol in this experimental model. From these results it would seem that 23-784 was a highly active beta-adrenoceptor blocking drug, potentially useful in the therapeutic induction of these drugs. The results presented in the next section, however, will show otherwise.

INTRINSIC SYMPATHOMIMETIC ACTIVITY (ISA)

Some beta-adrenoceptor blocking drugs themselves stimulate beta-adrenoceptors. This property is called intrinsic sympathomimetic activity (ISA). Figure 3 shows that the ISA of a drug like pindolol leads to a slight increase in resting heart rate of subjects in the supine position where sympathetic tone is very low. In the sitting position, when sympathetic tone is higher pindolol like propranolol (a drug without ISA) reduces heart rate, but to a lesser extent (3). If we now look at the results with 23-784 we see that this drug increases resting heart rate not only in the lying position (and to a greater extent than pindolol) but also in the sitting subject. The mean heart rate

[beats/min]

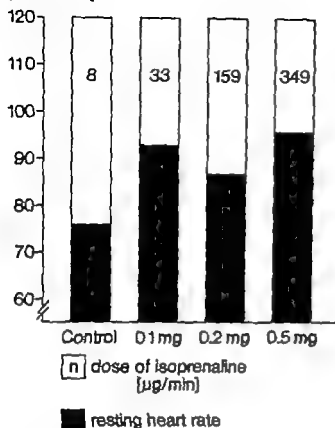


Fig 4 Resting heart rate in the lying position and dose of isoprenaline required for a heart rate of 120 beats/min before and 4½ hours after oral administration of 23-784 (mean $n=3$). The increase in resting heart rate shown on this figure is smaller than that seen in figure 3 the reason being that isoprenaline infusions could only be administered to 3 of the 4 subjects of figure 3 as in the fourth one heart rate had already reached a value above 120 beats/min without isoprenaline.

values reached after 4½ hours were 97 ± 10 beats/min in the lying and 98 ± 8 beats/min in the sitting position, compared with pre-drug values of 78 ± 7 beats/min and 85 ± 4 beats/min respectively. Figure 4 gives a synopsis of the effects of 23-784 on resting heart rate in the lying position and of the dose of isoprenaline required to increase heart rate to 120 beats/min. The figure demonstrates again the high beta-adrenoceptor blocking activity of 23-784 due to the higher resting values the large doses of isoprenaline required after oral administration of the drug were actually producing a much smaller rise in heart rate than in the control period. It may be concluded that pindolol possesses what might be called a 'well balanced' ISA which seems to be an advantage in so far as the drug neither leads to a great reduction nor to a great

increase in resting heart rate. In contrast 23-784 despite its high beta-adrenoceptor blocking potency exerts too much intrinsic sympathomimetic activity to be of use in the traditional indications of beta-adrenoceptor blocking drugs.

DURATION OF ACTION

Exercise-induced tachycardia is not only useful for investigating the potency but also the duration of action of a beta-adrenoceptor blocking drug. If equipotent submaximal doses of different drugs are administered, the experiment allows a comparison of the duration of action of these drugs.

Our experiments with pindolol (5 mg) and propranolol (100 mg) showed that the doses used of both drugs are about equiactive 2 hours after oral administration. The duration of action of pindolol however is clearly longer than that of propranolol. Whereas after 74 hours $16 \pm 4\%$ of the effect of propranolol remained, at this time pindolol showed $36 \pm 5\%$ of the maximum (2, 3).

The long duration of action of pindolol has been confirmed in several therapeutic studies in patients with essential hypertension where the drug was administered once or twice daily (6, 12).

BIOAVAILABILITY

The pharmacokinetics of beta-adrenoceptor blocking drugs have been reviewed by J. Meier (8) in the same issue of this journal. His paper shows that after oral administration of beta-adrenoceptor blocking drugs the amount of drug absorbed and also the amount of drug metabolized during the first passage through the liver (first pass effect) varies for the different substances. In order to know how much of the orally administered drug is available at the receptor site it seems therefore important to compare the effects of these drugs after oral and after intravenous administration.

In a study with oral and intravenous administration of different doses of pindolol beta-adrenoceptor blocking activity was determined on isoprenaline and exercise-induced tachycardia (4).

The results show that the activity found hours after oral administration is about the same as that seen 75 min after intravenous administration of the same dose. These results therefore support those of the pharmacokinetic studies showing a nearly complete absorption of pindolol (7, 8) and a small first pass effect in man (8).

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DISCUSSION

Lennart Hansson

You made a statement, Dr Aelg, that the sympathetic tone is very low in supine resting position, and for that reason there was an increase in heart rate with pindolol. If you had used a blocker with

out intrinsic stimulating activity you would then expect very little or no reaction in heart rate. But in fact, when you used propranolol, you showed a substantial reduction in heart rate. The disturbing fact was that the initial heart rate in the propranolol group was much higher than in the pindolol group. How do you explain that?

Walter Aelg

The group of subjects was the same, but even if you administer different drugs in randomized sequence to the same subjects, there always remains a variation of the resting values.

Lennart Hansson

Yes, but the heart rate at rest was definitely higher in the propranolol group

Walter Aelg

Resting heart rate in the supine position was 83 ± 3 beats/min before propranolol and 81 ± 4 beats/min before pindolol. This difference is not significant.

To your second point. Of course sympathetic tone is not zero in the lying position, but it is lower than in the sitting position. With a similar initial heart rate in the lying position the frequency decreased after propranolol and increased with pindolol. In the sitting position however resting heart rate was reduced both after propranolol and after pindolol.

Anders Veila

I would like to comment on the effect duration of pindolol versus propranolol. Patients never take single doses, but sustained medication. Therefore a more relevant comparison is to compare duration of effect after prolonged medication. We have done such a study comparing the effects of 160 mg of propranolol daily versus 10 mg of pindolol daily. The doses were given once daily and the patients were treated for one week. The effect on exercise induced tachycardia was studied 24 hours after the last dose. The quantitative effect of propranolol was higher than that of pindolol indicating that during sustained medication the duration of propranolol is longer than that of pindolol.

Nils Svedmyr

I want to raise one further question. It has been said that pindolol has bell-shaped dose-response curve. That means that higher doses produce less decrease in blood pressure, or that the lowering of a high dose can decrease the blood pressure even more. From what I have seen of the literature, this statement is based only on uncontrolled studies, and I have found no proof that the dose response curve for pindolol differs from other beta-blockers.

[beats/min]

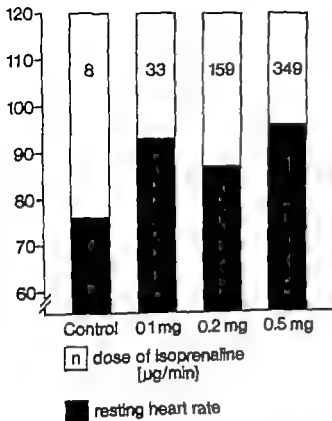


Fig 4 Resting heart rate in the lying position and dose of isoprenaline required for a heart rate of 120 beats/min before and 4½ hours after oral administration of 23-784 (mean $n=3$). The increase in resting heart rate shown on this figure is smaller than that seen in figure 3 the reason being that isoprenaline infusions could only be administered to 3 of the 4 subjects of figure 3 as in the fourth one heart rate had already reached a value above 120 beats/min without isoprenaline

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		Before therapy	β -blocker (therapy twice or 3-times daily)	Pindolol once daily		
				12 weeks	24 weeks	36 weeks
Group I n=16	Syst. BP	199 \pm 23	146 \pm 11	150 \pm 10	149 \pm 8	152 \pm 9
	Diast. BP	117 \pm 11	90 \pm 7	92 \pm 5	92 \pm 6	92 \pm 7
	Heart Rate		76 \pm 8	80 \pm 7	78 \pm 9	82 \pm 10
	Dosage mg			11.6	12.9	13.1
Group II n=14	Syst. BP	189 \pm 16	147 \pm 14	147 \pm 16	150 \pm 16	151 \pm 15
	Diast. BP	115 \pm 14	86 \pm 8	88 \pm 8	87 \pm 10	88 \pm 10
	Heart Rate		65 \pm 9	65 \pm 8	73 \pm 13	71 \pm 10
	Dosage mg			13.9	13.8	13.6
Group III n=14	Syst. BP	177 \pm 24	141 \pm 13	138 \pm 15	142 \pm 16	139 \pm 15
	Diast. BP	115 \pm 8	90 \pm 6	88 \pm 10	92 \pm 9	90 \pm 9
	Heart Rate		69 \pm 12	73 \pm 9	72 \pm 11	67 \pm 7
	Dosage mg			10.7	10.8	12.1
Total	Syst. BP	189 \pm 23	145 \pm 13	145 \pm 14	147 \pm 15	147 \pm 15
	Diast. BP	116 \pm 9	89 \pm 7	89 \pm 8	91 \pm 8	90 \pm 9
	Heart Rate		70 \pm 11	73 \pm 10	73 \pm 11	74 \pm 11
	Dosage mg			12.0	12.5	13.0

Table 2. Mean (\pm S.E.M.) supine blood pressure (mm Hg), heart rate (beats/min) and dosage of pindolol in 44 patients completing trial I. Group I, II and III represent patients previously treated with pindolol, alprenolol or propranolol respectively. Compared with the values during therapy 1, two or three times daily there were no significant differences for systolic and diastolic blood pressure or heart rate when patients were transferred to a once-daily dose of pindolol.

Design of the trial

The patients had to have a supine diastolic blood-pressure (DBP) of ≥ 105 mm Hg registered on at least two different occasions to be enrolled in the study. After that there was a placebo-period of three weeks. Patients, whose DBP fell below 105 mm Hg during the placebo-period were to be excluded from the study. The blood-pressure at the end of the placebo-period was recorded as the initial value in the calculations.

The active treatment started with 5 mg of pindolol given orally 1-8 a.m. The supine blood-pressure was measured at intervals of four weeks. The criterion for adequate control was considered to be a pressure of under 165/95. The dose of pindolol was increased—if necessary—in steps of 5 mg up to a single daily dose of 20 mg. Doses over 20 mg were not regarded as suitable in this study.

The period of active treatment covered four months. Thus, patients requiring 20 mg daily were observed for one month on that dose.

The blood-pressure was measured by the same person with the same mercury-sphygmomanometer for 10 minutes rest. The diastolic pressure was

	Age (years)		
	30—39	40—49	50—59
Men	2	5	3
Women		3	3

Table 3. Patient grouped according age and sex in study II. Sixteen patients.

Class-study II.	WHO I	WHO II	FH 0	FH I	FH II
Men	7	3	4	2	3
Women	5	1	3	2	1

Table 4. Patients grouped according to WHO and to funduscopy changes according to Keith-Wagener Barker.

of the sounds. The pressure was measured between 8—9 a.m. and the patients were instructed not to take the morning dose of pindolol that day. For statistics, Student's two-tailed t-test for paired differences was used.

Results

	n
Total no. in study	57
Age	
<40	9
40—59	40
≥60	8
Sex	
Men	37
Women	20
WHO Group	
I	32
II	25
Heart volume (ml/m ²)	
≤500 ml	43
>500 ml	14
Duration of hypertension	
< 1 year	5
1—2 year	31
3—5 year	10
6—10 year	7
>10 year	4

Table 1 Patients in study 1 grouped according to WHO-classification, duration of hypertension sex and age.

tension according to WHO-criteria, heart volume as well as the duration of hypertension is shown in Table 1

The patients were switched over to pindolol once daily beginning with 10 mg given in the morning. The pressure was checked at intervals of four weeks and if a satisfactory pressure-level, i.e. a diastolic pressure at or below 100 mm Hg was not maintained the dose was increased by 5 mg at each visit up to a single dose of 20 mg.

The blood pressure was measured after 10 minutes rest in the morning, and the patient was instructed not to take the tablets that morning. In this way it was ensured that 24 hours had elapsed since the last tablet intake.

The study was carried out for 36 weeks on the once daily regime with pindolol

Results

44 patients completed the trial and constituted the basis for the statistics. In four patients, a diastolic

pressure of 100 mm Hg could not be maintained even on 20 mg and those patients were excluded from the study. One patient died of a myocardial infarction after 8 weeks. In four patients side-effects were registered to such a degree that the treatment had to be abandoned. For three patients the reason was vertigo after tablet intake and for one gastrointestinal troubles in the form of diarrhoea. The effects on blood pressure are shown in Table 2. It shows the initial blood-pressure, the pressure after treatment with multiple doses of beta-blockers, i.e. at the start of the trial and the pressure after 36 weeks on a single daily dose of pindolol. It is seen that there is no statistical difference between the pressures before and after the trial. Compared with initial values, there were no significant differences for blood pressure or heart rate when patients were transferred to a once-daily dose of pindolol.

The duration of the study—36 weeks—is certainly long enough to rule out any possibility of a carry-over-effect from the previous treatment. However it is of course only shown, that it is possible to maintain an adequate pressure-control with pindolol once daily in patients already treated with multiple doses of beta-blockers. Therefore, a further study was carried out, starting with a single dose of pindolol from the beginning of the treatment of the hypertension.

STUDY II

Material and methods

Sixteen out-patients with mild to moderate previously untreated essential hypertension were selected for the study. They were all included in the routine examination scheme including ECG, funduscopy, chest x-ray, determination of electrolytes and creatinine in serum and analysis of urine for protein, glucose and blood-cells. If necessary further investigations were carried out, such as intravenous pyelogram, angiography and hormone analysis.

The age and sex distribution is shown in Table 3. In Table 4 the patients have been grouped according to the WHO-criteria of hypertension as well as to the funduscopy changes according to Keith Wagener Barker.

The usual contraindications for treatment with beta-blockers were considered.

scription of a long acting diuretic, which can also be administered once daily in order to preserve the benefits of a simple regime. This matter however needs further investigation. The material is too small to draw any certain conclusions regarding the side-effects. It is worth noting that no patients had sleep disturbances or nightmares. This is in line with earlier observations on once a day dosage (8) and perhaps these well known side-effects can be reduced when the tablets are given only in the morning.

No undesired beta-blocker-induced bradycardia was observed. As pindolol has an intrinsic sympathomimetic effect this complication is most unlikely to appear even on high doses of the drug. In a study like the present one, which is not performed with a strict double-blind technique the bias-risk has to be considered. However the aim has been to investigate a simple treatment and not to prove the hypotensive effect of pindolol, which is already documented. Furthermore it is debatable whether there is any possibility of performing a strict double-blind trial with beta-blockers, in view of their effect on the pulse rate both at rest and during work.

Sommarberg, the once daily dosage regime with pindolol seems to be a simple and effective way to treat mild to moderate hypertension with relatively few side-effects. It offers advantages, both for the patient and the physician, which are of great importance in view of the life-long duration of the therapy.

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DISCUSSION

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Lennart Sölvell

Do you think that the once daily administration of pindolol in the morning was the reason for the absence of sleep disturbances?

Göran Frithz

It is otherwise difficult to explain why in our study using once a day administration of pindolol, no one complained of sleep disturbances, when such complaints have been reported from studies using two or three doses a day

Table 5 Mean (\pm S.E.M.) supine blood pressure (mm Hg) before and after 4 months treatment Pindolol dose in 4th month Sixteen patients.

	Before treatment	After treatment	P
Systolic BP	181.2 \pm 7.1 (210 —170)	150.1 \pm 8.3 (165 —120)	<0.001
Diastolic BP	110.5 \pm 3.2 (120 —105)	91.0 \pm 3.0 (100 —80)	<0.001
Dose in 4th month	10 mg	15 mg	20 mg
WHO I patients	3	7	2
WHO II patients		1	3

No one had to be excluded due to a normalized pressure after the placebo period. Fourteen achieved the criteria set for satisfactory pressure-control, i.e. a pressure of <165/95 mm Hg. The two patients, who did not reach that level both belonged to the WHO II group and had a diastolic pressure of 100 mm Hg after four months, i.e. after one month on 20 mg pindolol. The results after four months treatment are shown in Table 5 as well as the dosages of pindolol at that time. It may be observed that the mean dosage of pindolol is somewhat lower for patients belonging to the WHO-I group than that for the WHO-II group.

Side-effects

Three patients reported side-effects, for which pindolol may have been responsible. One patient complained of palpitations, one of dizziness and one of slight nausea after taking the tablets. The first two patients were receiving 15 mg, the last one 20 mg. No one reported sleep-disturbances.

DISCUSSION

The study demonstrates the possibility of treating previously untreated hypertensive patients, starting with one single daily dose of pindolol.

It is reasonable to assume that this form of treatment should be restricted to milder forms of hypertension. From Table 5 it can be concluded that the smallest effective dose is 10 mg. It is almost certain, that this dose can be given directly without a previous running-in period with 5 mg. As the mode of tolerance to the single dose therapy was not known at the start of the study this design was chosen. However a further study now in progress indicates the possibility of starting even with 15 mg directly.

On the other hand it should perhaps also have

been possible to go further than 20 mg in one single dose. However it was judged as not advisable due to the possibility of subjective symptoms. Therefore after the study was ended it was considered preferable to treat the two cases who did not achieve a fully acceptable pressure-control on pindolol alone with a combination of a single daily dose of pindolol with one tablet of the fixed combination of 50 mg hydrochlorothiazide and 5 mg amiloride (Moduretic®) given simultaneously. On that combination both patients achieved a diastolic pressure of 90 mm Hg after one month's further treatment.

The blood-pressure was measured in the morning to obtain an interval of 24 hours from the last tablet intake. It could naturally be argued that it is of more interest to measure the pressure during the day in the activities of daily life. However it is well established that the effect of a peroral dose of pindolol reaches its peak after about 3–4 hours (1) so there is little reason to believe, that the pressure-control should be less satisfactory during the day. Furthermore it has recently been shown by Gordon (5) who treated patients who cooperated in measuring their own blood-pressure during the day that there were little differences in the over all pressure-control whether pindolol was administered once, twice or three times daily.

The difficulties of maintaining patients with known hypertension on continuous treatment are documented in many studies (2, 3). It is also known that the chances of missing tablet intakes increase as the dosage frequency increases (7). Therefore the administration of pindolol once daily can be useful in helping patients to comply with the prescribed treatment. If an adequate response is not obtained on a tolerable dose of the beta-blocker it is reasonable to suggest the compe-

scription of a long acting diuretic, which can also be administered once daily in order to preserve the benefits of a simple regime. This matter however needs further investigation. The material is too small to draw any certain conclusions regarding the side-effects. It is worth noting that no patients had sleep disturbances or nightmares. This is in line with earlier observations on once a day dosage (8) and perhaps these well known side-effects can be reduced when the tablets are given only in the morning.

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Summarizing, the once daily dosage regime with pindolol seems to be a simple and effective way to treat mild to moderate hypertension with relatively few side-effects. It offers advantages, both for the patient and the physician, which are of great importance in view of the life-long duration of the therapy.

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Future Development in the Field of Hypertension

Chairman Bernt Håkfelt

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Generally speaking the future developments in hypertension as applied to clinical medicine and health care will depend on how successful research will be in further exploring etiology and pathogenesis, and in producing efficient and safe diagnostic procedures and therapy. Of equal importance is the extent to which presently known and future diagnostic procedures and therapeutic means will be made available and used. These developments are partly the direct responsibility of the medical profession in a broad sense, but partly that of political decision-makers and administrators, for whom correct information based on knowledge and good advice from the medical expertise are of fundamental importance. The importance of the knowledge must be stressed in view of the present trends amongst politicians and others to overlook this absolute prerequisite for meaningful and successful health care, including preventive medicine.

Needless to say the future developments in the field of hypertension will be based on, and closely linked to the present and the past. Accordingly it might be appropriate to paint a background to the papers to follow by recalling some of the major events and progresses within hypertension research over the last three decades.

The wellknown American investigator Irvine Page at the end of the 1940:s proposed what he named the mosaic theory of hypertension.

According to this theory all the various components which influence blood pressure must be in equilibrium to maintain appropriate blood pressure and adequate tissue perfusion. If one factor changes and becomes dominant, the others will adjust accordingly. This theory has served as an inspiring and constructive model. The

various interdependent facets when placed in the corners of an octagon, make possible to visualize the great number of points at which blood pressure control can be interfered with and deranged—but also influenced therapeutically (Figure 1).

It might be worth while pointing out that the mosaic concept implies that hypertension principally is a disorder of blood pressure regulation which means that hypertension is induced via mechanisms, normally acting to maintain normotension. Accordingly the more we learn about physiological blood pressure and perfusion control and the various factors involved, the better we will understand the pathology of hypertensive disease.

It is gratifying to be able to state that the functional dependence and the integration of several of the facets covered in the octagon have been explored and even defined during the years after the presentation of the mosaic theory. In particular I would like to remind you of three major components:

1) In the middle of the fifties we witnessed the isolation, chemical identification and synthesis of aldosterone, the exponent for the physiologically so important mineralocorticoids but also the immediate cause of hypertension as documented in Conn's syndrome and other forms of primary aldosteronism. It has also become evident that aldosterone often is engaged secondarily in other forms of hypertension at various stages, such as renovascular hypertension, pheochromocytoma, primary reninism and malignant hypertension. From therapeutic point of view the discovery of aldosterone and the clarification of its role in disease led to the synthesis of widely used therapeutic agents such as the aldosterone antagonist spironolactone.

2) The discovery in 1898 by Tigerstedt and Bergman, that the kidney produces a hypertensive principle, remained a scientific enigma until Brown, Menendez, Helmer, Page and others demonstrated that renin is an enzyme inducing the production in the body of the very active vasoconstrictor peptide angiotensin which was isolated, structurally defined and synthesized, again events which happened in the middle of the fifties.

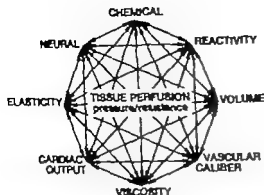


Fig. 1 The "Mosaic Theory"

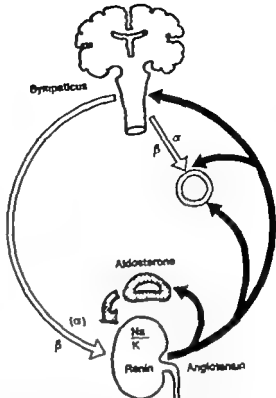


Fig. 2. Influence of angiotensin on blood pressure.

Further research has clearly established that angiotensin plays a physiologically important role as a constrictor agent under certain conditions and also that it plays a role as a stimulator of aldosterone production in health and disease. The hypertensive potential of renin-angiotensin has been convincingly demonstrated in cases of primary reninism and also in certain types of renovascular hypertension induced experimentally in animals and occurring spontaneously in man. There is also good evidence that angiotensin can influence blood pressure by increasing sympathetic activity via stimulation at the nerve endings and centrally (Figure 2).

3) Discoveries of fundamental importance have been made concerning the mediation of sympathetic function peripherally and centrally and with respect to the anatomical distribution of catecholaminergic neurons within the central nervous system. Suffice it to mention the concept of alpha and beta-adrenergic receptors published by Ahlqvist 1948 the demonstration of centrally located noradrenergic, dopaminergic and lately adrenergic neurons, and the presence centrally of alpha and beta-receptor mechanisms engaged in the regulation of blood pressure.

It has also become evident that not only does the renin-angiotensin system influence the activity of the sympathetic nervous system, as mentioned above, but that the sympathetic nervous system exerts a pronounced effect in stimulating renin secretion by the juxtaglomerular cells in the kidney. Accordingly sympathetic activity constitutes a determinant for the production of renin-angiotensin,

various interdependent facets, when placed in the corners of an octagon make possible to visualize the great number of points at which blood pressure control can be interfered with and deranged—but also influenced therapeutically (Figure 1).

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New Trends in Pharmacology

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Abstract. Among the factors influencing the arterial blood pressure the neural control through the autonomic nervous system is of paramount significance. The sympathetic division of this system holds a key position in this regard and its neural transmission mechanisms represent well established target for pharmacological interference aimed at lowering blood pressure. This presentation will first deal with the morphological and physiological basis for blood pressure control through the sympathetic nervous system and the various possibilities for antihypertensive drug action through this system. Special attention will be drawn to recent advances in catecholamine research which may offer new leads in the development of blood pressure lowering agents. A second topic in this review will be some remarks on other pharmacological principles for interference with vascular control apart from the sympathetic system, particularly the vasodilator principle.

1. CATECHOLAMINE MECHANISMS IN BLOOD PRESSURE REGULATION

As previously mentioned, the role of the peripheral sympathetic system and its neural transmission mechanisms in circulatory homeostasis is comparatively well recognized. The central nervous mechanisms determining the activity of the sympathetic system are considerably less understood. As will be shown later an increasing body of evidence indicates that central catecholamine (CA) neurons participate in cardiovascular control.

There is ample evidence for a transmitter function of the CA, dopamine (DA) and noradrenaline (NA) (5). These neurons have their cell bodies located in the lower brain stem (medulla oblongata, pons and mesencephalon) while their terminals are distributed to nearly all parts of the

brain and the spinal cord although with varying density. A number of CA pathways have been mapped, e.g. descending NA pathways from the medulla oblongata to the sympathetic centers in the spinal cord, ascending pathways from the brain stem to e.g. the hypothalamus and the preoptic area. This distribution is suggestive of a functional influence on central autonomic mechanisms. Within the brain stem are found short NA neurons which seem to innervate i.a. brain stem autonomic centers—again providing a morphologic background for central CA's regulating central autonomic function and hence the cardiovascular system.

Direct evidence for central CA mechanisms being involved in cardiovascular control has emerged from pharmacological studies utilizing CA amino acid precursors in combination with selective inhibitors of various steps in CA biosynthesis (20). The use of so called peripheral decarboxylase inhibitors has been of particular interest since they inhibit the enzyme activity only outside the central nervous system thus permitting a separation of central and peripheral actions. The CA precursor L-3,4-dihydroxyphenylalanine (L-dopa) produces a hypertensive response in the conscious rat (22). This is abolished or after a large dose of L-dopa converted into a hypotensive response when the enzyme dopa-decarboxylase is inhibited in peripheral tissues. The hypotensive effect is abolished when dopa-decarboxylase is inhibited both centrally and peripherally by other compounds entering the central nervous system, demonstrating that the lowering of blood pressure is mediated by an action of CA formed from L-dopa in the central nervous system. These results have been amply verified in several animal species (see 21) but no conclusive data from human experi-

resulting in effects on blood pressure regulation via a direct influence on the vessels but also via aldosterone production. Sympathetic amines, renin-angiotensin and aldosterone in reality have been found to function as a unit of physiological importance for blood pressure regulation but also contributing to hypertension of various kinds at various stages. Under certain conditions this functional integration actually constitutes a vicious circle.

As a result of new discoveries concerning renin-angiotensin and also sympathetic nervous function new agents have been produced of diagnostic and therapeutic potential such as converting enzyme inhibitor and competitive inhibitors of peripheral angiotensin action—not to mention all the compounds that have been made available to influence sympathetic function centrally and peripherally.

Using the complex mosaic theory as a background, I have tried to present some advances within a rather limited field but which actually represent a rather magnificent development.

We have touched upon neural, humoral and hormonal mechanisms of general importance for vasoconstriction salt balance volume regulation and peripheral resistance. No doubt, future research will lead to new discoveries within these areas. At the present time great interest is still focused on central and peripheral sympathetic mechanisms but in addition the field is expanding to include dopaminergic and serotonergic neurons the parasympathetic system and also the newly discovered field of putative transmitters of peptide nature such as enkephalin and substance P which undoubtedly will be found to play a role in central blood pressure regulation. In the meantime many investigators are engaged in the search for a sodium excretor the so called third factor which some believe to be related to bradykinin or other kinins or prostaglandins, known to be extremely active as vasodilators but also as promoters of sodium and water excretion. From discussions at this symposium it has been made clear that the hyperkinetic state of early essential hypertension mostly is *not* hyperkinetic but rather an expression of a metabolic disturbance the nature of which awaits exploration thus another area for future research.

In connection with theories on the mechanism of action of clonidine much interest has recently been paid to the relevance of so-called presynaptic receptor mechanisms. The first indications of such mechanisms came from studies on the effects of NA receptor blocking agents on CA turnover: it was found that the utilization of CA appeared to be increased in the presence of CA receptor blocking agents (11). It was also soon found that CA receptor agonists, e.g. clonidine, caused a decrease in CA utilization (6). These observations have been confirmed both in peripheral and in central nervous CA neurons. Apparently the CA released by neuronal activity once it reaches a threshold concentration in the synaptic gap activates presynaptic receptors, probably located on the nerve ending which then trigger a negative feedback mechanism which slows down synthesis and release of the CA transmitter (3, 12). There is now ample evidence for the existence of presynaptic receptors or autoreceptors as they are perhaps more aptly designated in both peripheral and central nervous CA neurons. They may be of the α -type, mediating a retardation of CA synthesis and release but probably also of the β type which may be activated by low concentrations of CA and lead to an increase in the CA transmitter output (3, 12). To complicate the matter further there are also indications of presynaptic muscarinic inhibitory receptors in at least peripheral CA nerve endings. The ubiquitous prostaglandins have also been reported to influence CA release: prostaglandins of the E series inhibit NA release in peripheral tissues (37).

The functional significance of these autoreceptors in drug action is by no means clarified but it is of interest that clonidine has a much higher affinity to these receptors than to the "classical" post-synaptic α -receptors in the peripheral sympathetic system. Also, certain biochemical presynaptic clonidine effects occur in the same dose range as that required to lower blood pressure in animals (4). It may therefore be that clonidine, in certain dose ranges, owes its hypotensive properties to an action on presynaptic CA receptors. On the other hand, clonidine facilitates the vagally mediated cardiodepressor reflex and this action has been shown to be independent of NA release from central neurons, clearly showing direct stimulation of postsynaptic NA receptors (14).

Thus, this issue is far from settled and further more, the clinical implications are obscure although it may be that it has bearing on interactions between clonidine and other drugs acting at central CA receptors. Neuroleptic agents of the phenothiazine type exemplify this: animal studies suggest that they may counteract the hypotensive effect of clonidine but clinical pharmacological studies to this effect have not been performed. The tricyclic antidepressants also have profound effects on central CA mechanisms and, again, animal experiments that show antagonistic effects towards the antihypertensive effect of clonidine are available but the clinical counterpart is lacking (see 35). The observation that α -adrenergic blocking agents such as tolazoline completely abolish the hypotensive effect of clonidine in man (9) is a clinical counterpart to the pharmacological studies previously mentioned indicating a central nervous action of clonidine on α -receptors in man.

From practical point of view centrally acting antihypertensive drugs like methyldopa and clonidine offer certain advantages, particularly when compared to peripheral sympathetic nervous blocking agents such as guanethidine and bethanidine. Thus, methyldopa and clonidine cause considerably less orthostatic side-effects than the peripherally acting drugs and other signs of sympathetic blockade e.g. failure of ejaculation are rarely seen in patients receiving methyldopa and clonidine (for references see 15, 19). However both these drugs produce a sedative effect, which represents a major drawback in their clinical usage. This effect is probably correlated to an interference with central CA mechanisms involved in the maintenance of (i.e. wakefulness (26) and may be inherent in the principle of action of these drugs.

New developments in the field of centrally acting antihypertensive drugs include several structural analogues of clonidine; so far all drugs in this category subjected to clinical trials have displayed a similar profile of side-effects as clonidine, i.e. a sedative effect. Some examples of such agents are guanabenz (30) and BS 100-141 (23). It would appear that more information must be sought with regard to structure-activity relationships of these central NA receptor agonists. There exists the possibility that the receptors mediating a decrease in sympathetic activity may have dif-

ments seem to be available. It has been suggested that the occurrence of orthostatic hypotension during L-dopa treatment of patients with Parkinson's disease is of central origin and analogous to the hypotensive effect observed in animal experiments. However the use of combinations of L-dopa and peripheral decarboxylase inhibitors in treating Parkinsonian patients does not always reduce the incidence of orthostatic reactions.

The hypotensive action of L-dopa has been subject to further analysis and results from several different research groups indicate that the effect is mediated by activation of central NA receptors, most likely located in the lower brain stem (21). The occurrence of NA nerve terminals in this region as well as its key position in blood pressure regulation should be noted in this connection. Thus, the nucleus of the tractus solitarius and the dorsal nucleus of the vagus, both of which participate in the central mediations of baroreceptor reflexes, show a high density of CA terminals. Furthermore, bilateral lesions of these structures result in a severe hypertension and alterations in CA metabolism in the central nervous system (14).

2. CENTRAL CATECHOLAMINE MECHANISMS AND ANTIHYPERTENSIVE DRUG ACTION

Studies aimed at elucidating the mode of action of L- α -3,4-dihydroxyphenylalanine (methyldopa) provided the earliest indications of central CA regulation of blood pressure. This subject has been reviewed in detail elsewhere (19). In summary the most convincing arguments against the so-called "false transmitter" theory came from studies of the interaction between methyldopa and a peripheral decarboxylase inhibitor which was shown to prevent the synthesis of false transmitters from methyldopa in the peripheral sympathetic system while not influencing the antihypertensive action of this drug. On the other hand, this action is abolished following decarboxylase inhibition in both the peripheral and the central nervous system. These observations gave clear indication that the antihypertensive action of methyldopa is mediated by its CA decarboxylation products in the central nervous system. Subsequent analysis using i.e. inhibitors of the formation of NA from DA have demonstrated that methyl-NA is implicated apparently acting by direct stimulation of NA re-

ceptors. As in the case of L-dopa, these receptors are probably located in the brain stem (20, 21).

Little attention has been paid to the possible relevance of these observations in the clinical use of methyldopa in hypertensive patients. This is somewhat surprising since non-toxic peripheral decarboxylase inhibitors are currently available, being used in conjunction with L-dopa in the treatment of Parkinson's disease. Apart from the indirect evidence provided by the various well known central nervous side effects of methyldopa in man only one report is available which indicates a central site of attack (31). The experiment, performed on a single patient, is a direct parallel to the animal studies referred to above studying the influence of peripheral decarboxylase inhibition on the antihypertensive action of methyldopa. As in animal experiments, this was found to be unaffected in spite of a pronounced decrease in the formation of methyldopa decarboxylation products. From the pharmacodynamic point of view a controlled study in a larger group of patients would be necessary before the results from animal experiments can be extended to man.

There is general agreement among pharmacologists that the antihypertensive effect of clonidine is mediated largely by an action in the central nervous system (35). In animal experiments, clonidine is also an effective agonist at central nervous NA receptors and agents known to block these receptors also prevent the cardiovascular effects of clonidine (35). It is therefore conceivable that the hypotensive action of clonidine results from an activation of central NA receptors. These receptors are usually classified as belonging to the α type since clonidine has α -adrenergic effects in peripheral tissues and since the NA receptor antagonists which block the central actions of clonidine also are peripheral α -adrenergic blocking agents. As in the case of L-dopa and methyldopa the receptors mediating the hypotensive response to clonidine seem to be located in the lower brain stem. However there are also indications that clonidine influences blood pressure at suprabulbar levels in the brain. Thus, actions of clonidine in the posterior hypothalamus may contribute to the hypotensive effect and there is also evidence that hypothalamic and/or cortical NA mechanisms influenced by clonidine may mediate blood pressure increasing actions (34).

In connection with theories on the mechanism of action of clonidine much interest has recently been paid to the relevance of so-called presynaptic receptor mechanisms. The first indications of such mechanisms came from studies on the effects of NA receptor blocking agents on CA turnover. It was found that the utilization of CA appeared to be increased in the presence of CA receptor blocking agents (11). It was also soon found that CA receptor agonists, e.g. clonidine, caused a decrease in CA utilization (6). These observations have been confirmed both in peripheral and in central nervous CA neurons. Apparently the CA released by neuronal activity once it reaches a threshold concentration in the synaptic gap, activates presynaptic receptors, probably located on the nerve ending which then trigger a negative feedback mechanism which slows down synthesis and release of the CA transmitter (3, 12). There is now ample evidence for the existence of presynaptic receptors or autoreceptors as they are perhaps more aptly designated in both peripheral and central nervous CA neurons. They may be of the α -type, mediating a retardation of CA synthesis and release but probably also of the β type which may be activated by low concentrations of CA and lead to an increase in the CA transmitter output (3, 12). To complicate the matter further there are also indications of presynaptic muscarinic inhibitory receptors in at least peripheral CA nerve endings. The ubiquitous prostaglandins have also been reported to influence CA release, prostaglandins of the E series inhibit NA release in peripheral tissues (32).

The functional significance of these autoreceptors in drug action is by no means clarified but it is of interest that clonidine has a much higher affinity to these receptors than to the "classical" post-synaptic α -receptors in the peripheral sympathetic system. Also certain biochemical presynaptic clonidine effects occur in the same dose range as that required to lower blood pressure in animals (4). It may therefore be that clonidine, in certain dose ranges, owes its hypotensive properties to an action on presynaptic CA receptors. On the other hand, clonidine facilitates the vagally mediated cardiodepressor reflex and this action has been shown to be independent of NA release from central neurons, clearly showing a direct stimulation of postsynaptic NA receptors (24).

Thus, this issue is far from settled and further more, the clinical implications are obscure although it may be that it has bearing on interactions between clonidine and other drugs acting on central CA receptors. Neuroleptic agents of the phenothiazine type exemplify this: animal studies suggest that they may counteract the hypotensive effect of clonidine but clinical pharmacological studies to this effect have not been performed. The tricyclic antidepressants also have profound effects on central CA mechanisms and, again, animal experiments that show antagonistic effects towards the antihypertensive effect of clonidine are available but the clinical counterpart is lacking (see 35). The observation that α -adrenergic blocking agents such as tolazoline completely abolish the hypotensive effect of clonidine in man (9) is a clinical counterpart to the pharmacological studies previously mentioned indicating a central nervous action of clonidine on α -receptors in man.

From a practical point of view centrally acting antihypertensive drugs like methyldopa and clonidine offer certain advantages, particularly when compared to peripheral sympathetic neurons blocking agents such as guanethidine and bethanidine. Thus, methyldopa and clonidine cause considerably less orthostatic side-effects than the peripherally acting drugs and other signs of sympathetic blockade, e.g. failure of ejaculation are rarely seen in patients receiving methyldopa and clonidine (for references see 15, 19). However both these drugs produce a sedative effect, which represents a major drawback in their clinical usage. This effect is probably correlated to an interference with central CA mechanisms involved in the maintenance of i.e. wakefulness (26) and may be inherent in the principle of action of these drugs.

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ferent characteristics from those causing the sedative effect, allowing for a greater selectivity in agonist action. Future research in this field could follow such lines.

The β -receptor blocking agents represent a novel approach to anti-hypertensive therapy and their mechanism of action is a challenge to pharmacologists. Various peripheral actions such as baroreceptor resetting, cardiac effects, decreased renin secretion have been held responsible for their anti-hypertensive property but do not provide a satisfactory explanation (review in 1). There is by now a fairly large number of studies which suggest that β -blocking drugs may act by influencing CA mechanisms.

First, it has been demonstrated in animal experiments that propranolol diminishes the effect of vasoconstrictor nerve stimulation without decreasing the sensitivity infused NA (2). Secondly the release of NA from sympathetic vasoconstrictor nerves is decreased after β receptor blockade (1). Third there is a significant decrease in the vasoconstrictor nerve function in spontaneously hypertensive rats after long-term treatment with propranolol and metoprolol (27). All these observations could be explained by a blockade of pre-synaptic receptors of the β type in peripheral sympathetic nerves (27). As previously mentioned these receptors seem to mediate an increase in the release of NA and their blockade would then of course diminish the output of NA. Clinically it has been reported that the urinary output of NA is reduced during therapy with a β -blocker (16).

Finally it should be mentioned that a number of investigators have found evidence that central nervous effects of β receptor blocking drugs may participate in mediating the decrease in blood pressure (13). This theory is largely based upon experiments involving local administration of β -blocking agents into the brain tissue or the cerebral ventricles and therefore not immediately transferable to the clinical situation. Somewhat more convincing are the observations that intravenous infusion of propranolol into conscious animals leads to a reduction in the electrically recorded activity in sympathetic nerves (25). Considerable more work is required before this question is settled.

With regard to future developments in the field of β -receptor blockade there is little doubt that

the cardioselective (β_1) receptor blocking agents have come to stay. Although there is evidence that the subdivision of β -receptors is less strict than previously thought clinical experience clearly shows that β_1 -selective blockers offer considerable advantages in for example treating hypertensive patients with bronchial obstruction. These blockers also have the haemodynamic advantage over the non-selective type that β_1 -selective blockade leaves the endogenous β_2 -receptor mediated vasodilator effects unopposed, which may contribute to the blood pressure lowering effect of the β_1 -selective blockers (29). However administration of salbutamol to practolol treated hypertensive patients gave no further fall in blood pressure (7). Non-selective blockers with intrinsic beta-mimetic activity could theoretically lead to a somewhat similar haemodynamic pattern and there are clinical observations which support this theory (Atterhög, Dunér & Pernow this symposium).

On the other hand, this type of non-selective blockers will produce a smaller decrease in cardiac output than a blocker without intrinsic activity (29). Since a majority of the hypertensive population have an increase in peripheral vascular resistance, there is an obvious need for a vasodilator component in the therapeutic regimen. This may of course be accomplished by the use of vasodilator drugs in a combination therapy with β -blocking agents but practical complexities call for drugs combining β -blocking and vasodilatory properties. At least one such drug, labetalol (AH 5158) has been given fairly extensive clinical trial (33). This agent combines non-selective β -receptor and α -receptor blocking effects, thus influencing both cardiac output and vascular resistance.

Although orthostatic reactions do not seem to have been a major problem in the use of labetalol it should be pointed out, that blockade of the α -receptors always implies a potential risk of orthostatic blood pressure fall. It appears that careful titration of dosage decreases this risk in the case of labetalol. Another approach to increase the antihypertensive effect of β -blocking drugs would be to combine β -blockade with a selective vasodilatory action on resistance vessels, such compounds have been synthesized but are still largely at the pre-clinical stage of development. At present time the prescribing physician has to resort to giving

the β -blocking and the vasodilator drug as separate formulations.

3. VASODILATOR DRUGS

Vasodilator drugs for use in antihypertensive treatment should influence resistance vessels selectively any dilating effect on capacitance vessels will imply a theoretical possibility for orthostatic reactions. As mentioned above, α -receptor blockade carries this risk and can never be used as the sole principle in antihypertensive therapy. Dilatation of the resistance vessels will lower total vascular resistance and, therefore, blood pressure, but will also trigger a number of reflex adjustments aimed at counteracting the fall in blood pressure. These mechanisms include increase in cardiac output, increased secretion of renin and hence a state of hyperaldosteronism, all of which seriously limit the usefulness of vasodilator drugs as single agents in the treatment of hypertension (cf. Hansson, this symposium). It has been claimed that some of these agents are less liable than others to cause e.g. sodium retention (as a consequence of the previously mentioned reflex mechanism), but a critical evaluation shows no major differences in this respect. Some of the currently available vasodilators are not exerting a single action, i.e. dilatation of the resistance vessels. Thus, prazosin which has received extensive clinical trials also tends to produce a slight α -receptor blockade, particularly at higher dosage and leading to orthostatic side-effects. It has been shown, that the problem may be partly overcome by careful titration of dosage and employing the smallest possible doses, particularly at the onset of prazosin treatment (18).

An interesting class of pharmacological agents may constitute a new principle for the therapy of arterial hypertension, although these drugs are currently used for other purposes. These drugs interfere with the transport of calcium ions at one or several stages in the handling of these ions by various cells. Vascular smooth muscle, like striated muscle and myocardial muscle is highly dependent on the availability of calcium ions, which serve to mediate the coupling between excitation and contraction. Calcium ions, liberated from intracellular storage sites take part in the interaction between the contractile elements of the muscle and pharmacological agents interfering with these processes

may profoundly influence the excitation-contraction coupling. This has been demonstrated for both myocardial and vascular preparations in vitro (17) and probably represents the basic mechanism underlying the effects of these drugs in man. Verapamil is perhaps the best known representative for this class of drugs: calcium antagonism will lead to e.g. a decrease in myocardial oxygen consumption and a beneficial effect in angina pectoris. In addition, verapamil influences the conduction system of the heart, notably the specialized, calcium-dependent mechanisms in the atrio-ventricular node providing a basis for its action on certain types of cardiac arrhythmias. There are now several reports indicating a therapeutically useful effect of verapamil in cases of arterial hypertension (10) but as expected with a high incidence of effects on cardiac conduction. Another recently introduced powerful calcium antagonist, nifedipine, has also been reported to be effective in lowering pressure in hypertensive patients (8) but again, complicating actions from the myocardium are to be expected. Since the most distal resistance vessels which seem to function as "pace-maker" cells in the maintenance of myogenic vascular tone are probably more dependent on calcium availability it is theoretically possible to envisage a dose of e.g. nifedipine which only affects resistance vessel tone and leaves myocardial function intact. However it should be emphasized that this principle for antihypertensive is still at its early experimental stage and experience from clinical materials is very much limited. The problem of possible reflex adjustments resulting from the lowering of vascular resistance has not been evaluated so far and it should be pointed out that a concomitant therapy with β -blocking drugs may present hazards, these drugs also affect intracellular calcium movements resulting in a synergistic action with the calcium antagonistic agents. Serious complications (e.g. circulatory collapse or cardiac arrest) have been reported after combinations of β -blocking drugs and verapamil. Much additional evaluation is required before allowing any conclusions regarding the position of calcium antagonists in the treatment of hypertension.

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Dopamine Receptor Stimulants in Hypertension

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Until comparatively recently interest in dopamine was based largely on its position as the metabolic precursor of noradrenaline. Carlsson's (3) finding that the corpus striatum, which contains practically no noradrenaline, is extremely rich in dopamine, suggested that dopamine might have a transmitter function of its own in the central nervous system. There is now considerable evidence that this is so.

Dopamine is also found in the adrenal medulla, lung, liver, gut, kidney and carotid body (2, 6, 8, 14-16). Although its physiological significance in these tissues is not yet clear the fact that the amounts found vary from one tissue to another and sometimes exceed the levels of noradrenaline, suggests that dopamine may have a transmitter function in the periphery also.

Dopamine has a number of pharmacological actions which differ from those of noradrenaline and

which are clearly unrelated to its role in noradrenaline synthesis (for review see references 7). Dopamine is a potent cardiac stimulant. Although this action is mediated by activation of β -adrenoceptors, increases in myocardial contractile force can be obtained at low doses without a simultaneous increase in heart rate. Heart rate is increased only at relatively high doses. This is in contrast to the β -adrenoceptor stimulant, isoprenaline, which induces parallel increases in contractile force and heart rate (Table 1). Dopamine also stimulates vascular α -adrenoceptors, resulting in increases in blood pressure. After blockade of this effect with an α -adrenoceptor antagonist, a depressor effect is unmasked which is only partially inhibited by β -adrenoceptor blocking agents. The residual fall in blood pressure has been shown to be due principally to dilation of mesenteric and renal vessels. It is unaffected by ganglion blocking

Table 1. Effects of catecholamines on the heart and blood vessels in dogs and man. Δ = increase, ∇ = decrease, \leftrightarrow = no change.

	Catecholamine	Effects on			
		Heart rate	Contractile force	Heart rate	Contractile force
ADRENERGIC	Isoprenaline	Increases Δ	Increases Δ	Increases Δ	Increases Δ
	Dopamine	Increases Δ	Increases Δ	Increases Δ	Increases Δ
	Noradrenaline	Increases Δ	Increases Δ	Increases Δ	Increases Δ
	Epinephrine	Increases Δ	Increases Δ	Increases Δ	Increases Δ
ADRENERGIC	Isoprenaline	Increases Δ	Increases Δ	Increases Δ	Increases Δ
	Dopamine	Increases Δ	Increases Δ	Increases Δ	Increases Δ
	Noradrenaline	Increases Δ	Increases Δ	Increases Δ	Increases Δ
	Epinephrine	Increases Δ	Increases Δ	Increases Δ	Increases Δ
ADRENERGIC	Isoprenaline	Increases Δ	Increases Δ	Increases Δ	Increases Δ
	Dopamine	Increases Δ	Increases Δ	Increases Δ	Increases Δ
	Noradrenaline	Increases Δ	Increases Δ	Increases Δ	Increases Δ
	Epinephrine	Increases Δ	Increases Δ	Increases Δ	Increases Δ

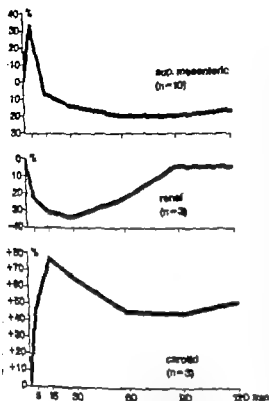


Fig. 3 Mean percentage changes in vascular resistance induced by intravenous administration of Sandoz 27-403 10 μ g/kg. in the anesthetized dog (chloralose urethane).

vessels. This probably resulted from the abrupt fall in perfusion pressure and consequent reduction in blood flow which occurred during the first few seconds following injection of the compound. Thereafter resistance was decreased for a period of at least two hours. A greater reduction in resistance occurred in the renal vessels. The relatively rapid recovery probably reflected intrarenal autoregulation. In contrast to these effects, resistance increased in vessels in the region supplied by the common carotid artery. The effects observed in the femoral vessels were variable. Decreases in flow in the carotid vascular bed may have been due to redistribution of blood into the mesenteric and renal vessels or to reflex constriction occurring in vessels in which dopamine receptors are not present. Direct stimulation of α -adrenoceptors by the drug can be ruled out, the compound does not increase blood pressure in spinal

animals, neither does it increase tone in isolated arterial strips.

The decreases in blood pressure and resistance evoked by Sandoz 27-403 are not diminished by the β -adrenoceptor blocking drug, pindolol, but are abolished by pretreating dogs with the dopamine receptor blocking drugs, haloperidol (20) and ergometrine (1).

It has been shown that ligation of the renal, coeliac, superior and inferior mesenteric arteries abolishes the blood pressure lowering effect of dopamine (5). It was this finding that led to the conclusion that dopamine receptors are present mainly in the vascular beds supplied by these major arteries. Sandoz 27-403 and dopamine failed to lower blood pressure after ligation of the major vessels in the abdomen. This confirmed Ebble's (5) findings and also added weight to the concept that the blood pressure lowering effect of Sandoz 27-403 is due entirely to stimulation of dopamine receptors.

We have performed a series of similar experiments with the ergot alkaloid, bromocriptine (2-bromo- α -ergokryptine-mesylate). There is considerable evidence suggesting that this drug stimulates dopamine receptors within the central nervous system (4, 11, 12). Postural falls in systolic blood pressure have been reported in normotensive patients undergoing therapy with high doses of bromocriptine (9, 17, 18) and there are indications that the drug may lower blood pressure in hypertensive patients (13) and increase sodium excretion (unpublished observations). It seemed reasonable to assume that these effects occur as a result of stimulation of peripheral dopamine receptors.

Volkman and Goldberg (19) examined this possibility but were unable to demonstrate a dilator effect in renal vessels following intra-arterial injections of doses of bromocriptine one thousand times the minimal effective dose of dopamine. We have confirmed the lack of effect following both intra-arterial and intravenous injections. However if the drug is administered slowly by the intravenous route, falls in blood pressure can be obtained from a dose of only 6 μ g/kg (Figure 4). It will be noted that almost one hour is required before the maximum effect is achieved, in contrast to Sandoz 27-403 which exerts its maximum effect within 5 min of administration. The reduc-

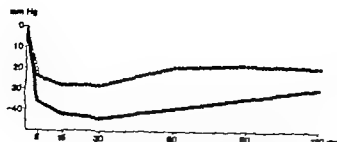


Fig 1 Effect of intravenous administration of Sandoz 27-403 on mean systemic blood pressure in the anaesthetized dog (chloralose-urethane). Mean reductions in pressure obtained in response to 2.5 µg/kg 0000 (n=3) and 10 µg/kg 0000 (n=11).

agents, atropine, antihistamines and pretreatment with reserpine, but can be selectively attenuated by the dopamine receptor blocking agent, haloperidol (20)

The extensive work of Goldberg and his colleagues has led to the concept that the action of dopamine on renal and splanchnic vessels is due to stimulation of receptors which are specific for the amine. They have shown, in addition, that dopamine not only increases total renal blood flow but changes the cortical medullary perfusion ratio, increases glomerular filtration rate and enhances sodium excretion. It is its action on the kidney which distinguishes it from other catecholamines. The unique profile of activity of dopamine on the cardiovascular system and on renal function has led to clinical trials of dopamine for the treatment of shock, congestive heart failure, oliguric renal failure, drug intoxication and hypertension (7)

In a study performed in patients with severe hypertension (15) infusions of dopamine 1–1.5 µg/kg/min raised mean blood pressure by 10 mmHg. After administration of the α -adrenoceptor blocking drug phenoxybenzamine (1 mg/kg) the dopamine infusions decreased blood pressure by an average of 77 mmHg. Cardiac output was increased by 2.6 litres/min, total peripheral resistance fell by 50% and heart rate increased by 16 beats/min. Creatinine clearance did not diminish during the period of reduced blood pressure (15). As a result of this study Goldberg (7) suggested that a substance which selectively stimulates dopamine receptors, that is, which lacks α and β -adrenoceptor stimulant activity which possesses a long duration of action and is orally



Fig 2 Effect of intravenous administration of Sandoz 27-403 on heart rate in the anaesthetized dog (chloralose-urethane). Mean changes in rate obtained in response to 2.5 µg/kg 0000 (n=3) and 10 µg/kg 0000 (n=11). Same animals as in figure 1

absorbed might prove to be an effective antihypertensive agent.

The dopamine derivative Sandoz 27-403 (N-bis [6[3,4-dihydroxyphenyl] ethylamino] benzyl] hexamethylethylenediamine tetrahydrobromide) fulfils many of these criteria. It induces long-lasting stimulation of vascular dopamine receptors, it does not stimulate α -adrenoceptors and has only weak affinity for β -adrenoceptors. It fails to meet the last requirement in that absorption from the gastrointestinal tract is poor.

Sandoz 27-403 lowers blood pressure in conscious, anaesthetized dogs from a dose of 2.5 µg/kg given intravenously. The effect is reproducible from one animal to another and lasts for a minimum of two hours (Figure 1). An important feature of the action of this compound is that, unlike most other antihypertensive agents which relax vascular smooth muscle, the reduction in blood pressure does not evoke reflex tachycardia. Relatively short lasting increases in heart rate occur in response to a dose of 2.5 µg/kg, but such an effect is rarely observed when a higher dose is given despite the fact that the blood pressure fall is greater. On the contrary heart rate almost always decreases (Figure 2).

The blood pressure lowering effect of Sandoz 27-403 seems to be entirely due to dilation of vessels in the mesenteric and renal vascular beds. Blood flow in the superior mesenteric artery together with that in a renal, carotid or femoral artery was measured using electromagnetic flow probes. Regional vascular resistance was calculated as blood pressure divided by flow (Figure 3). Intravenous administration of 10 µg/kg resulted in a transient (apparent) increase in resistance in the mesenteric

bromocriptine in comparison to apomorphine, d-amphetamine and L-DOPA. *Br J Pharmacol* 56: 59, 1976.

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I wonder if Dr Henning would like to comment on the central nervous action of beta-blocking agents. There was a very interesting recent paper by Lewis and co-workers where they recorded the sympathetic nerve activity. Their results provide evidence, that at least some of the anti-hypertensive effect of the beta-blocking agents might be mediated through the central nervous system.

Maria Henning

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Berns Håkfelt

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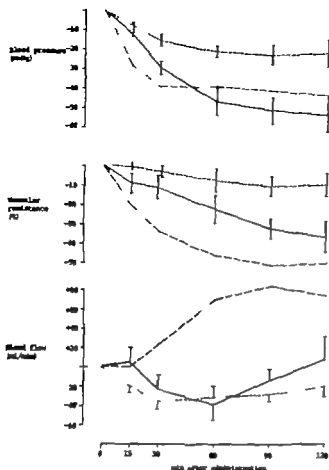


Fig 4 Effects of intravenous administration of bromocriptine in the anaesthetized dog (chloralose urethane). Mean changes (\pm S.E.M.) in blood pressure and resistance and blood flow in the superior mesenteric vascular bed induced by 6.25 μ g/kg ($n=5$) — 12.5 μ g/kg ($n=5$) and ——— 25 μ g/kg ($n=2$).

tion in blood pressure which occurred in response to 25 μ g/kg was not significantly different from the response to 12.5 μ g/kg. At both doses, blood pressure fell to approximately 70 mmHg. We have observed that a dopamine receptor stimulant rarely depresses blood pressure to a level below this in normotensive animals.

Resistance in the superior mesenteric (Figure 4) and renal vessels is decreased by bromocriptine, whereas that in the carotid vascular bed is unchanged or increased. The effects of bromocriptine, like those of Sandoz 27-403 are abolished by haloperidol and ergometrine confirming an effect on dopamine receptors. The hypotensive response to the drug can also be eliminated by ligating the major vessels in the abdomen. This suggests not only that the fall in blood pressure is the con-

sequence of a vasodilator effect in the vascular beds supplied by these vessels, but also that it is a peripheral event. However there is evidence suggesting the existence of dopaminergic pathways within the central nervous system mediating hypotensive responses (10). In view of the potent stimulant effects of bromocriptine on dopamine receptors in the central nervous system, an additional action on central dopaminergic pathways concerned in blood pressure control cannot be excluded as a contributory factor in the hypotensive action of the drug.

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Matts Henning

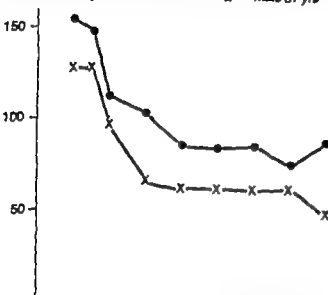
From our experiences, in acute experiments, we would expect an increase. But here I understand it was chronic treatment.

Bernt Håkfelt

With reference to the very interesting studies presented by Miss Clark concerning the hypotensive effect of dopamine analogues, I would like to pre-

Nonadrenaline
urine nmol/day

● Female 38 yrs.
X Male 37 yrs



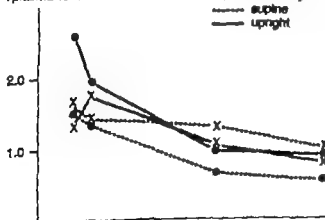
sent a slide showing some recent results obtained in our department. During treatment of 9 acromegalic patients, Dr Anders Nilsson has followed catecholamines in plasma and urine before and during increasing doses of the dopamine agonist bromo-alpha-ergocryptine (CB 154 an ergot derivative). So far determinations have been completed only with respect to two of the patients. The figures demonstrate a marked decrease in the noradrenaline levels in plasma and urine. These figures might be of interest in view of the recently reported hypotensive effect of bromo-alpha-ergocryptine.

Barbara Clark

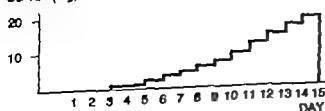
I think this is of particular interest since it implies that there is a reduction in sympathetic tone. Experiments have been performed by Dr Scholtysek in our laboratories in which he injected bromocriptine into the cerebral ventricles in cats. He has shown a reduction in blood pressure and heart rate at a dose which is ineffective when given intravenously. It seems that bromocriptine may well have a dual action peripheral and on the central nervous system.

Noradrenaline
/plasma nmol/l

● Female 38 yrs
X Male 37 yrs.
--- supine
— upright



Daily dose
CB 154 (mg)



The Prevention of Hypertension

Gösta Tibblin and Carl-Gunnar Eriksson

From the Department of Social Medicine, University of Umeå,
Umeå, Sweden

Abstract. Our way to prevention is to find a list of traits known to be predictors of elevated blood pressure. This list of predictors offers means for the early identification of susceptible persons. Years of experience in preventive work indicate that such identification is always useful for developing preventive programmes, since it gives a focus for action (3).

Most of the predictors show possible ways in which action could be directed towards prevention of hypertension and reduction of elevated blood pressure.

We will focus on salt, control of obesity, physical exercise, and medication. We would like to discuss preventive aspects of hypertension and the possibility of treating with other methods than drugs.

In the United States there is a development of an effective national programme to identify, evaluate, and treat 20 million or more hypertensive persons (1-5). The same procedure is going on in Sweden in order to take care of our quarter of a million hypertensive persons.

These national programmes are certainly a huge and complex task, requiring a great deal of careful hard work. But, as Jerry Stamler pointed out "that challenge should not blind us to a basic fact: in the long run, the ultimate solution of the hypertension problem is primary prevention, not case finding and drug treatment, important as they are now" (4).

An extensive range of epidemiological studies have searched for predictors of elevated blood pressure (6-14).

The list of predictors as you see in Table 1 is stable.

Positive family history
First-born child

• Overweight—weight gain

• Glucose intolerance

• High sodium intake

• Social stress
Rapid heart rate
Peptic ulcer
Renal disorders
Hearing loss

Table 1. Predictors of hypertension.

SALT

The importance of salt in the pathogenesis of hypertension was emphasized as early as 1904 by Ambard and Beauvard. Guyton (15) indicated the overriding importance of the relationship between blood pressure and renal functional capacity to handle excess sodium in determining the presence or absence of hypertension. The crucial role of extracellular fluid volume in the reduction of blood pressure with diet therapy and diuretics has been emphasized by many authors (16).

It is a striking fact that hypertension is not found in many less civilized populations nor does the blood pressure rise with age in these populations (13, 14, 16). Obviously there must be some environmental factor which accounts for the difference. From our point of view this is of great interest. Hypertension is preventable in some places! In the April issue 1976 of *Circulation*, Ed Fabsitz, in an article titled Salt, volume and the prevention of hypertension (16), has summarized the literature about blood pressure in populations from widely different parts of the world including New Guinea, Malaysia, Eastern Island, American

Basin, San Blas Islands of Panama, rural Uganda and the Kalahari Desert of Africa.

In every epidemiological study of this type, when salt is not added to the diet hypertension is low or absent, and when salt is used the prevalence of hypertension is high.

When we move towards the studies of western populations—Dawber in Framingham (17) and Miall in England (18) we will not find any correlation between sodium intake and blood pressure. The ordinary western diet is about 5 to 15 grams of salt per day.

But if the daily intake of salt averages about 25 grams per day as in the northeastern district of Japan the prevalence of hypertension in the fifth decade would be found to be 30 to 40%.

We have summarized the findings from epidemiological studies of the relationship between blood pressure and salt surveyed in Freis article in a table showing critical levels of sodium intake and the processes of developing hypertension (Table 2).

RELATIONSHIP BETWEEN DIETARY SODIUM EXTRACELLULAR FLUID (ECF) AND BLOOD PRESSURE

Salt ingested in the diet is distributed predominantly to the extracellular space. Excess amounts of salt and water are eliminated primarily through the kidneys. Guyton has linked ECF and blood pressure in the following way:

The common dominator in the development of

any chronic elevation of blood pressure is the need for the excretion, that is to prevent chronically expanded ECF. The level of blood pressure required to produce the diuresis will depend upon the ability of a particular kidney to excrete an excess of sodium which varies from one individual to another. The importance of dietary sodium in the maintenance of the ECF has been known for many years. Using Kempner rice diet, (19) which is very low in sodium (8 mEq) the ECF fell 12 per cent, there was a fall in blood pressure.

The inference from these considerations with respect to the prevention of hypertension would now seem fairly obvious. The level of ECF which we call normal is probably an expanded one compared to that of our primitive forebears. This condition has arisen because civilized man has developed his diet and a return to natural, unsalted foods will by contracting ECF reduce the stimulus to develop hypertension that presently exists in our society.

OBESITY

Extensive evidence (11) demonstrates that over weight in youth, young adulthood and middle age and gains in weight in the years from young adulthood into middle age are associated with an increased risk of hypertension (Table 3). However high blood pressure does not develop in every obese person nor in every weight gainer. Almost certainly factors other than simple overeating are

Table 2. Critical levels of sodium intake related to prevalence of hypertension from epidemiological studies.

Sodium per day	Prevalence of hypertension
<10 mEq	Hypertension is absent. No rise with age
10—70 mEq	Few hypertensives
70—350 mEq	15% hypertensives. Rise with age
>350 mEq	30% of adults hypertensives

Table 3. Relationships between relative weight slope of weight curve over 20 years and prevalence of hypertension.

Relative body weight	Prevalence of hypertension
<100	128
100—112	229
113—	368
Weight slope 20 years	Development of hypertensives
Negative weight slope	0.4
Weight slope 0.5—1.4	178
Weight slope 1.5 or >	500

in work. Chasing et al. (21) in an extensive review of the literature examined 19 experimental and clinical observations on the effect of weight reduction. They found a general trend toward reduction of blood pressure. They conclude by saying that weight reduction lowers blood pressure in a considerable proportion of obese persons.

The mechanism behind the frequent coexistence of overweight and hypertension is little known.

Many obese persons have increased cardiac output. Alexander (22) postulated that an increased stroke volume is required to pump blood through an expanded vascular bed in the obese person. It is possible that the increased cardiac output influences the autoregulation of the resistance vessels so that the peripheral fluid volume is also increased in obese persons and this mechanism may therefore be working in obesity as well as in high salt intake.

PHYSICAL ACTIVITY

The trait which can be linked with low physical activity is rapid heart rate. There is no clear evidence showing that low physical activity per se is a risk factor for developing hypertension.

Physical training generally tends to decrease the resting heart rate with a decreased or unchanged cardiac output (23, 24).

Small and insignificant effects of physical training on blood pressure have been observed in young subjects (25, 26) but significant reductions in blood pressure by physical training have been obtained with sedentary 40 years old men (27) and normal older men (28). Studies on effects of physical training in hypertension are few. One study (29) of four hypertensive subjects on a four week conditioning programme showed a decrease in arterial peripheral resistance but no reduction of blood pressure. Four other studies obtained reductions in resting blood pressure (30-33). Boyer and Ketch (30) observed significant reductions in diastolic pressure (12 mm Hg) and systolic pressure (13.5 mm Hg) in 32 essential hypertensives while there was a drop only in diastolic pressure (6 mm Hg) in 22 normotensive middle-aged men after a controlled exercise programme for six months. Sannerstedt and collaborators (32) reported normalization of blood pressure after 6 weeks of physical conditioning in five subjects with latent hypertension of the hyperkinetic type. Reductions

of systolic and diastolic blood pressure at rest and during submaximal work following a six-month conditioning programme have been reported by Choquette and Ferguson (33) in both normotensive and borderline hypertensive men.

The drop in pressure suggested that borderline hypertensives may derive an even greater benefit from such a programme.

PRACTICED MEDITATION

Practiced meditation is an approach which influences such predictors as "spikes" of high blood pressure, rapid heart rate and social stress.

Several studies have during the last years shown significant reduction of blood pressure using meditational techniques both in established hypertension and borderline hypertension (34-43).

The technique developed by Beary et al. (44) involves four components: a mental device, a passive attitude, decreased muscle tones and regular practice. There is a constant stimulus of a silently repeated secret sound or word, called a mantra. The purpose of this repetition is to shift one's attention from logical, externally oriented thought. The person should sit in a comfortable position so that minimal muscular work is required and he is instructed to practice the technique for two daily 20-minute periods, usually before breakfast and before dinner.

The meditational technique is associated with several physiological changes which are easily reproduced (45-47).

The response is believed to be associated with decreased sympathetic nervous system activity and is hypothesized to be the counterpart of Cannon's emergency reaction, popularly called the fight or flight response. The decreased sympathetic nervous system activity may also carry over into nonmeditational periods during the day and result as we have heard in the decreased blood pressures noted in these investigations mentioned.

SUMMARY

The four approaches which are rational in terms of our present knowledge in order to prevent hypertension are:

1. Consumption of a habitual diet with low salt content.
2. Weight-reduction through proper nutrition and exercise.

Basin San Blas Islands of Panama rural Uganda and the Kalahari Desert of Africa.

In every epidemiological study of this type, when salt is not added to the diet hypertension is low or absent, and when salt is used the prevalence of hypertension is high.

When we move towards the studies of western populations—Dawber in Framingham (17) and Mill in England (18) we will not find any correlation between sodium intake and blood pressure. The ordinary western diet is about 5 to 15 grams of salt per day

But if the daily intake of salt averages about 25 grams per day as in the northeastern district of Japan the prevalence of hypertension in the fifth decade would be found to be 30 to 40%

We have summarized the findings from epidemiological studies of the relationship between blood pressure and salt surveyed in Freis article in a table showing critical levels of sodium intake and the processes of developing hypertension (Table 2).

RELATIONSHIP BETWEEN DIETARY SODIUM EXTRACELLULAR FLUID (ECF) AND BLOOD PRESSURE

Salt ingested in the diet is distributed predominantly to the extracellular space. Excess amounts of salt and water are eliminated primarily through the kidneys. Guyton has linked ECF and blood pressure in the following way

The common dominator in the development of

any chronic elevation of blood pressure is the need for the excretion, that is to prevent chronically expanded ECF. The level of blood pressure required to produce the diuresis will depend upon the ability of a particular kidney to excrete an excess of sodium, which varies from one individual to another. The importance of dietary sodium in the maintenance of the ECF has been known for many years. Using Kempner rice diet, (19) which is very low in sodium (8 mEq) the ECF fell 12 per cent, there was a fall in blood pressure.

The inference from these considerations with respect to the prevention of hypertension would now seem fairly obvious. The level of ECF which we call normal is probably an expanded one compared to that of our primitive forebears. This condition has arisen because civilized man has developed his diet and a return to natural, unsalted foods will by contracting ECF reduce the stimulus to develop hypertension that presently exists in our society

OBESITY

Extensive evidence (11) demonstrates that over weight in youth, young adulthood and middle age, and gains in weight in the years from young adulthood into middle age are associated with an increased risk of hypertension (Table 3). However high blood pressure does not develop in every obese person nor in every weight gainer. Almost certainly factors other than simple overeating are

Table 2. Critical levels of sodium intake related to prevalence of hypertension from epidemiological studies.

Sodium per day	Prevalence of hypertension
<10 mEq	Hypertension is absent. No rise with age
10—70 mEq	Few hypertensives
70—350 mEq	15% hypertensives. Rise with age
>350 mEq	30% of adults hypertensives

Table 3 Relationships between relative weight, slope of weight curve over 20 years and prevalence of hypertension.

Relative body weight	Prevalence of hypertension
<100	128
100—112	229
113—	368
Weight slope 20 years	Development of hypertensives
Negative weight slope 0.4	114
Weight slope 0.5 1.4	178
Weight slope 1.5 or >	500

et work. Chasing et al. (21) In an extensive review of the literature examined 19 experimental and clinical observations on the effect of weight reduction. They found a general trend toward reduction of blood pressure. They conclude by saying that weight reduction lowers blood pressure in a considerable proportion of obese persons.

The mechanism behind the frequent coexistence of overweight and hypertension is little known.

Many obese persons have increased cardiac output. Alexander (22) postulated that an increased stroke volume is required to pump blood through an expanded vascular bed in the obese person. It is possible that the increased cardiac output influences the autoregulation of the resistance vessels so that the peripheral fluid volume is also increased in obese persons and this mechanism may therefore be working in obesity as well as in high salt intake.

PHYSICAL ACTIVITY

The trait which can be linked with low physical activity is rapid heart rate. There is no clear evidence showing that low physical activity per se is a risk factor for developing hypertension.

Physical training generally tends to decrease the resting heart rate with a decreased or unchanged cardiac output (23, 24)

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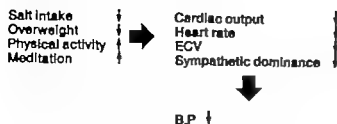


Fig 1 Measures and mechanism in primary prevention of hypertension.

- Regular frequent exercise to achieve preliminary cardiac fitness, with consequent slowing of heart rate and parasympathetic "dominance"

No proof is available that these measures achieve the primary prevention of hypertension but they are reasonable inferences from available data.

A controlled trial among overweight persons with family history of hypertension and rapid heart rate is certainly worthwhile doing.

The recommended measures are all safe and carry considerable side-benefits in increased well being and increased fitness.

The whole programme can in a controlled trial also be recommended for application to mild hypertensives and borderline hypertension. Such a study is in the planning stage in Umeå Sweden.

Lester Breslow has formulated. It's what you do hour by hour day by day that largely determines the state of your health, whether you get sick, what you get sick with and perhaps when you die. This programme influences our way of living and gives the subject full responsibility for his treatment.

Finally I have translated my speech into the symbols used in this seminar in order to increase the understanding of my thesis and summarize my presentation (Figure 1)

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DISCUSSION

Lennart Hansson

I agree with Gösta Tibblin as regards the beneficial effect of reducing salt intake on blood pressure in the population but I think one should also say a word of warning against it. The effect you can expect in the individual patients is really minimal. There are some studies done by for example Anton Amery who managed to reduce salt intake by 50% or more from approximately 180 mmol per day to 80 mmol, and the effect on blood pressure in these hypertensive patients was in the order of 3–5 mm Hg. I think you need such a drastic reduction of salt intake, that it is not sufficient just to take away the added salt. You also need salt free bread, saltfree milk and things like that, which are available for example in Belgium.

Gösta Tibblin

I have only given a review of the literature and I think we should give this approach a chance in a controlled trial. It is not necessary to have the salt consumption we have. You can certainly live with 1/2 g salt a day but it means certainly that we must start showing an interest in this.

Bengt Schersten

In the population, we have people with low blood pressure and high blood pressure and there are about 50% of each if you consider the median value. That would mean that if the salt intake has any meaning for hypertension, those with the lowest blood pressure should have a low intake, and those with the highest blood pressure should have a high intake. This is not the case there is no such difference in the intake of salt in our population at least in the southern part of Sweden.

Gösta Tibblin

I think this is very clear and as I mentioned, Dawber and Miall made the same finding. When you

are eating 5—15 grams a day there is no relationship between salt and high blood pressure. You must reduce the intake to a much lower level. Nobody has found any relationship between salt intake and blood pressure in such a high salt eating population as you are referring to.

Göran Berglund

I would like to point out that decreasing weight and heart rate by diet and physical training for example would not result in a large drop of blood pressure. The correlation coefficient between blood pressure and heart rate and between blood pressure and weight is only about 0.2. That means that the determination coefficient would be around 0.05 which means that only 5% of the variation in blood pressure can be explained by variation in heart rate and variation in weight. So you cannot expect very much drop in blood pressure. If you change these variables.

Gösta Tibblin

I am sure that one can expect a considerable drop in blood pressure. I refer here to a lot of studies showing significant decreases in blood pressure following, for example physical activity salt reduction meditation or reduction in overweight.

A NEW PARALLEL PLATE DIALYSER

I Development and Testing of Membrane Support

by S. Dawids and C. Boe

II Description of Design and in Vitro Performance

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Acta Medica Scandinavica

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A New Parallel Plate Dialyser

1 Development and Testing of Membrane Support

S. Dawids and C. Boe

From Medical Department P, Division of Nephrology, University Hospital, Rigshospitalet, Copenhagen and Department of Mechanical Engineering, Technical University of Denmark, Lyngby, Denmark

ABSTRACT The pattern of membrane support in the dialysate flow path of a dialyser is one of the most important parameters for high utilization of the membrane-area. 27 different injection moulded membrane support patterns were designed to approximate a pattern with the best overall properties. Only 15 of the patterns had acceptable properties in regard to 1) efficiency, 2) compliance, 3) flow resistance, 4) membrane rupture level, and 5) air retention at low flow rates. A pattern with parallel interrupted ridges perpendicular to the direction of dialysate flow showed the best overall results although differences were marginal between the best patterns. The chosen support pattern was approximately 35% better in chloride clearance at low dialysate flow and approximately 200% better at high flow than the conventional kilt pattern.

INTRODUCTION

In the design of a dialyser a number of criteria must be considered which are not only related to the performance of the item but also dictated by the available and suitable materials, their biological acceptability and their price. A pleasing appearance and easiness of handling in the clinic must also be considered. Although these points may heavily influence the final design, the performance is of major importance for the clinical applicability. Performance depends on two important independent factors, the chosen membrane and the membrane support, the latter being of special importance as it determines the inside topography of the blood layer, the degree of "abraded" membrane area and the submembranous dialysate mixing.

Through the years some efforts have been made to find an efficient all round membrane support

which could meet the requirements for a cheap industrial production. Among available methods only the injection moulding process seems to be applicable in practice giving relative freedom of the design, good tolerance of dimensions, rapid manufacturing etc. Other methods can be considered and have actually been used (e.g. profile stamping, punching, casting) but have proven to be too costly and complicated to handle in mass production.

Routinely used dialysers have mainly had three types of membrane supports. One consisting of parallel grooves as used in the Steggs-Leonard dialyser (13) and later in the Kilt dialyser (10, 14). The other consisting of multiple points, pyramids or cones as demonstrated in e.g. the Dialung (2, 3, 4, 7). The third is the mesh type of support initially used in coil dialysers (8, 11).

Although reportedly very efficient (primarily because of better blood geometry) a porous support made from nickel foam initially described by Babo and Grimmerud (1) proved unsuitable for routine use as the surface was too readily compressed leaving sharp edges which in turn injured the membrane. The concept of using porous support material led to subsequent testing (9, 12, 15) of other commercially available materials, but the results were generally disappointing indicating that only special purpose membrane supports could meet the requirements.

It is commonly accepted that a membrane support primarily should possess the following properties.

1) Efficient bracing of the membrane to avoid sagging especially under high transmembrane pressure gradients.

2) High utilization of the effective membrane

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constant concentrations and after each run the salinity is automatically replenished from the reservoir containing concentrated saline (3) via a solenoid valve (2) on the lid of the test chamber. Superfluous liquid in the chamber is simultaneously drained away. The test bench contains a control unit (not shown in figure) with a 24-position shift relay by which combinations of 6 different preset dialysate flows (Q) and 4 different transmembrane pressures (TMP) can be established by a pressure-regulation efficient pump (15). The measured concentration and times are recorded with a Servogor recorder Type RES 11 with event marker.

For testing with organic compounds (creatinine, urea) some of the test solution is continuously recirculated through the cuvette (6) of an LKB Uvicord Type 470-1A.

If one membrane support had a better efficiency for chloride compared with another support pattern, it was assumed that this would reflect a better efficiency for other substances under uniform testing conditions. To ascertain this measurements were made with chloride, creatinine and uric acid on 3 support patterns.

Although the assumption proved correct, the results showed that the differences in efficiency because smaller as the membrane permeability to the substance decreased. The chloride efficiency was an easy and reproducible measurement and because of the relative high permeation factor small differences were more clearly reflected in the results. Thus only chloride efficiency results are presented for the membrane support. Each membrane support was run at least 6 times through the 4-powder program, achieving 144 measurements at flow values of 50, 100, 150, 200, 250 and 300 ml/min and TMP values of 50, 105, 300 and 500 mmHg (a few support had a high resistance to dialysate flow which prevented measurement at 50 mmHg TMP because the membrane was pressed upward into the chamber and subsequently torn by the impeller). Deviation in measurements were found to be about 2% and thus the experimental error lies within approximately 8% because concentration errors are magnified in the calculations which uses differences in concentrations.

For the mathematical modelling of the system the following assumptions were made:

- 1) The removal of substance takes only place through the membrane area.
- 2) The volume of the test solution is constant

during the test. (The actual loss during a run never exceeded 10-15 ml or 1% of the volume.)

3) The removal of substance from the test solution through the membrane per unit time is equal to the amount of substance leaving the dialysate compartment.

The relation between the dialysis time (T) the dialysate flow (Q) and the diffusion through the membrane is expressed by

$$T = \frac{\ln}{B} \quad B = \frac{Q(1 - \exp[-(Lm \cdot A)/Q])}{Vol}$$

where Lm is diffusion constant dependent on the diffusion through the membrane and the boundary layers and A is the membrane area. The permeation factor $P = Lm \cdot A$ of the equipment can be derived from this equation:

$$P = \ln \left(1 - \frac{Vol \ln 2}{Q \cdot T} \right) Q$$

As the present experimental set-up is designed to ensure that conditions above the membrane and in the membrane itself are kept constant variations in the permeation factor (P) will solely reflect variation in the restriction to free diffusion on the dialysate side caused by unstirred boundary layers, shading by the support pattern and by air bubbles, as well as by uneven distribution of dialysate flow. P remains unaffected by simple flow-dependent variations in concentration gradients and subsequent changes in mass-transfer across the membrane. Determination of P thus permits a comparison not only of the performance of different support patterns but also of the same support at different flows.

Results of measurements of T are given in man-

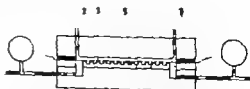


Fig. 2. Schematic presentation of test cell for evaluation of flow distribution, bubble retention and flow capacity of the membrane support. 1. Sealing strip, 2. Transparent lid, 3. Blood compartment, 4. Dialysate compartment, 5. Dialysis membrane, 6. Membrane support pattern, 7. Base, 8. Distribution channels for blood and dialysate, 9. Spacer.

area i.e. a low degree of "shading" by the membrane support largely independent of variations in transmembrane pressure gradients

3) Low dialysate flow resistance to avoid high entry blood pressure in the dialyser

4) Continuous mixing of fluids to reduce the phenomenon of unstirred layers

5) High resistance to membrane rupture thereby avoiding clinical "haematuria" during dialysis also with a fragile membrane

6) Low retention of air bubbles within the support pattern

These properties are however partially conflicting and consequently the resulting membrane support will always represent a compromise between merits

In practice it is thus not possible to design the ideal membrane support through calculations mainly because of the multifactorial considerations indicated above. The present paper reports on experimental results on different membrane support patterns one of which is applied in a new parallel plate dialyser (5)

MATERIALS AND METHODS

Pattern construction

Evaluation of a membrane support must be performed on areas comparable at least to a single plate of a future dialyser to check flow distribution, air bubble trapping, flow resistance etc. Areas of such sizes are not only costly but also time consuming to produce. To overcome this problem an experimental injection mould was constructed to hold a 30×30 mm replaceable square matrix with any given support pattern. Multiple squares were then made from each matrix and carefully glued together to form a suitable area. Each new membrane support pattern could thus be made within a short time.

All support patterns were tested with the same batch of Cuprophane® 150 PM (from Enka Glanzstoff Wuppertal) which was the thinnest commercially available dialysis membrane at the time.

Efficiency testing

Determination of efficiency was performed by measuring the time necessary to dialyse the salinity of a given volume of test solution from one preset concentration to another (in this case half the initial concentration) against "dialysate" consisting of

tapwater heated to 38°C. A shorter dialysis time corresponds to a higher efficiency. With a known volume of test solution which is recirculated dialysed against single pass dialysate flow, the relation between clearance and dialysis time can be expressed by the equation

$$CL = \frac{\text{Vol} \ln(C_0/C_t)}{T}$$

where CL is the clearance (in ml/min), Vol is the volume of the test solution (in ml), C_0 is the initial concentration, C_t is the terminal concentration, and T is the time in minutes.

Efficiency was measured on a test bench which is shown schematically in Fig. 1. It consists of a cylindrical chamber (12) with a volume of test solution of 1300 ml which is thoroughly mixed by an impeller (11) turning at constant speed near the bottom of the chamber. The bottom consists of the dialysing membrane (10) which rests on the membrane support (13) fitted in a base plate. The base contains entry and drain channels (7) for dialysate and is fitted with manometers (9, 14) at the dialysate connections. Inside the chamber an inductive conductivity meter (1) is mounted. This instrument has been described in an earlier paper (6) and has the prominent feature of being practically insensitive to air bubbles in the water and on the surface of the transducer.

The measurement is made between two preset

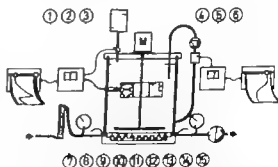


Fig. 1 Diagram of test bench for efficiency testing. 1 Conductivity transducer. 2, Solenoid valve remotely controlled. 3 Reservoir with brine to replenish the salinity before a new run. 4 Synchronous motor. 5 Small roller pump. 6 Ultraviolet absorption sensor. 7 Inlet of dialysate. 8 Flow meter with flow regulation valve. 9 Entrance pressure reading. 10 Membrane. 11 Impeller mixing the dialysate at constant speed. 12, Saline volume (1200 ml). 13 Membrane support on test. 14 Exit pressure reading. 15 Effluent pressure regulation pump.

constant concentrations and after each run the salinity is automatically replenished from the reservoir containing concentrated saline (3) via a solenoid valve (2) on the lid of the test chamber. Superfluous liquid in the chamber is simultaneously drained away. The test bench contains a control unit (not shown in figure) with a 24-position shift relay by which combinations of 6 different preset dialysate flows (Q) and 4 different transmembrane pressures (TMP) can be established by a pressure-regulation effluent pump (15). The measured concentration and times are recorded with a Servogor recorder Type RES 11 with event marker.

For testing with organic compounds (creatinine, urate) some of the test solution is continuously recirculated through the cuvette (6) of an LKB Unicord Type 470-1A.

If one membrane support had a better efficiency for chloride compared with another support pattern it was assumed that this would reflect a better efficiency for other substances under uniform testing conditions. To ascertain this measurements were made with chloride, creatinine and urate on 5 support patterns.

Although the assumption proved correct the results showed that the differences in efficiency became smaller as the membrane permeability to the substance decreased. The chloride efficiency was an easy and reproducible measurement, and because of the relative high permeation factor small differences were more clearly reflected in the results. Thus only chloride efficiency results are presented for the membrane supports. Each membrane support was run at least 6 times through the 24-position program, achieving 144 measurements at flow values of 40, 100, 150, 200, 250 and 300 ml/min and TMP values of 50, 105, 300 and 500 mmHg (a few supports had high resistance to dialysate flow which prevented measurements at 50 mmHg TMP because the membrane was pressed upwards into the chamber and subsequently torn by the impeller). Deviation in measurements were found to be within 7% and thus the experimental error lies within approximately 8% because concentration errors are magnified in the calculations which uses differences in concentrations.

For the mathematical modelling of the system the following assumptions were made:

1) The removal of substance takes only place through the membrane area.

2) The volume of the test solution is constant

during the test. (The actual loss during a run never exceeded 10–15 ml or 1% of the volume.)

3) The removal of substance from the test solution through the membrane per unit time is equal to the amount of substance leaving the dialysate compartment.

The relation between the dialysis time (T) the dialysate flow (Q) and the diffusion through the membrane is expressed by

$$T = \frac{\ln 2}{B} \quad B = \frac{Q(1 - \exp(-Lm A/Q))}{Vol}$$

where Lm is diffusion constant dependent on the diffusion through the membrane and the boundary layers and A is the membrane area. The permeation factor $P = Lm A$ of the equipment can be derived from this equation:

$$P = \ln \left(1 - \frac{Vol \ln 2}{Q T} \right) Q$$

As the present experimental set-up is designed to ensure that conditions above the membrane and in the membrane itself are kept constant, variations in the permeation factor (P) will solely reflect variation in the restriction to free diffusion on the dialysate side caused by unstirred boundary layers shading by the support pattern and by air bubbles as well as by uneven distribution of dialysate flow. P remains uninfluenced by simple flow-dependent variations in concentration gradients and subsequent changes in mass-transfer across the membrane. Determination of P thus permits a comparison not only of the performance of different support patterns but also of the same support at different flows.

Results of measurements of T are given in min-

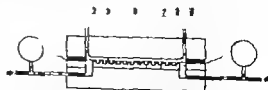


Fig. 2. Schematic presentation of test cell for evaluation of flow distribution, bubble retention and flow capacity of the membrane support. 1. Scaling strip, 2. Transparent lid, 3. Blood compartment, 4. Dialysate membrane, 5. Dialysate compartment, 6. Membrane support pattern, 7. Base, 8. Distribution channels for blood and dialysate, 9. Spacer.

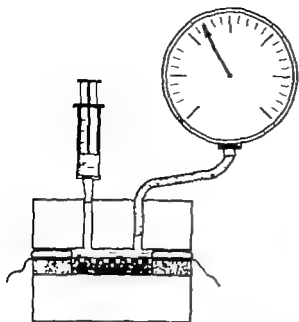


Fig 3 Schematic presentation of pressure cell for evaluation of compliance and rupture level of membrane on different membrane supports. The cell is also used for casting of membrane topography

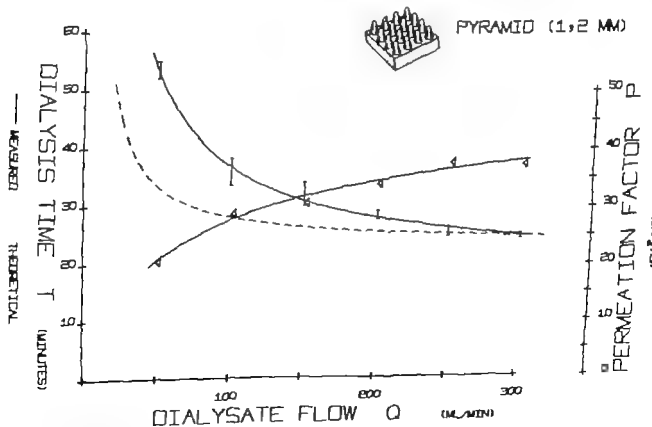


Fig 4 Graph representing the gradual improvement of performance with increasing flow. For further explanation see text

utes as a function of dialysate flow (ml/min) is computerdrawn curve as e.g. shown in Figs. 4 and 6 which also illustrate the calculated values P (ml/min). A high value of P indicates an efficient membrane support and in Fig. 7 the values are given in columns at two different values of Q (50 ml/min) a high column indicating a high value of P .

Flow distribution and air bubble retention could not be tested in the equipment shown in Fig. 1 in the equipment illustrated in Fig. 2 which has a transparent lid and a test area of $90 \times 150 \text{ mm}^2$.

Flow distribution was evaluated both in the "blood" compartment and in the dialysate compartment by bolus injection of colour into the flowing liquid. All patterns were tested in this way and only patterns showing uniform distribution were submitted for further evaluation of efficiency etc.

Air bubble retention within the membrane support pattern was measured on photographs by placing a grid over the picture and counting the squares with and without air. In practice the variability of multiple tests by this method was approx $\pm 10\%$.

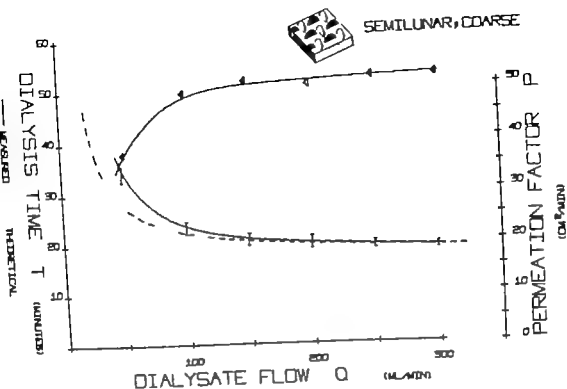


Fig 5 Graph representing sudden improvement of performance with increasing flow. For further explanation see text.

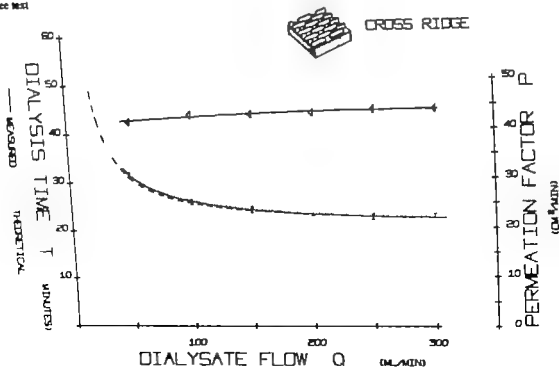


Fig 6. Graph representing an almost constant per

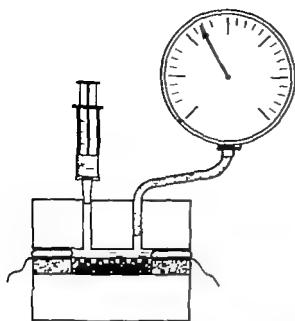


Fig 3 Schematic presentation of pressure cell for evaluation of compliance and rupture level of membrane on different membrane supports. The cell is also used for casting of membrane topography

utes as a function of dialysate flow (ml/min) in a computerdrawn curve as e.g. shown in Figs. 4, 5 and 6 which also illustrate the calculated values for P (ml/min). A high value of P indicates an efficient membrane support and in Fig. 7 the values are given in columns at two different values of Q (50 and 300 ml/min) a high column indicating a high value of P .

Flow distribution and air bubble retention could not be tested in the equipment shown in Fig. 1 but in the equipment illustrated in Fig. 2 which has a transparent lid and a test area of $90 \times 150 \text{ mm}^2$.

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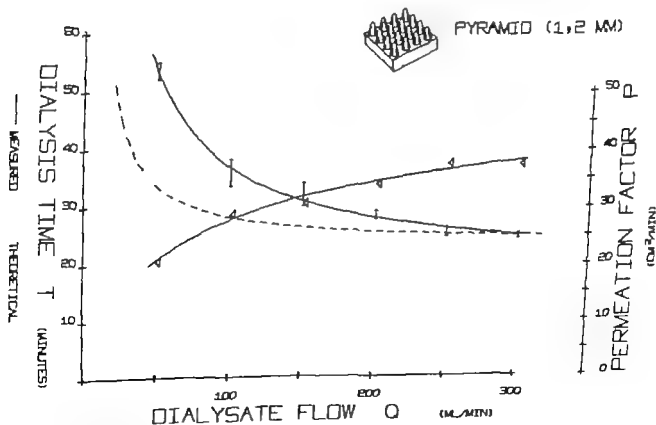


Fig 4 Graph representing the gradual improvement of performance with increasing flow. For further explanation see text

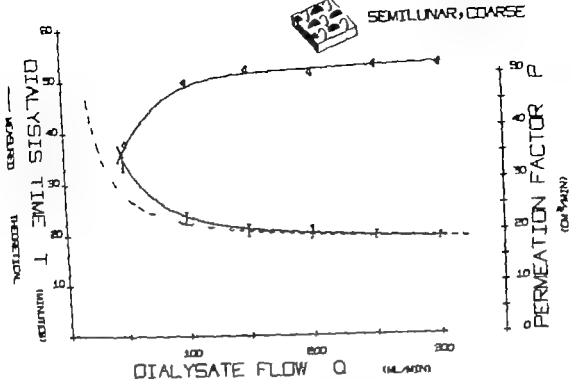


Fig 5 Graph representing a sudden improvement of performance with increasing flow. For further explanation see text.

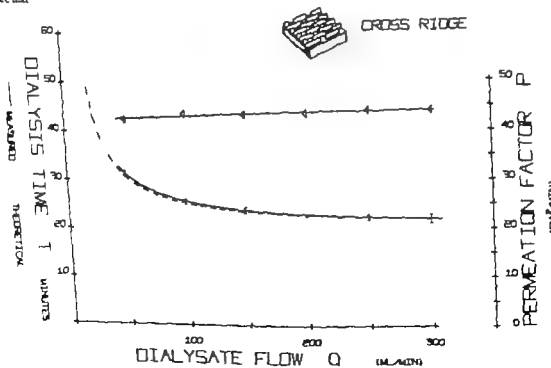


Fig 6 Graph representing an almost constant performance over a large flow range. For further explanation see text.



Fig 7 Diagrammatic representation of the evaluated properties of 15 membrane supports. High columns indicate good performance in the particular parameter. The differences

between the best patterns are marginal. Pattern no. 13 was chosen for support in the dialyser.

any given membrane support. Measurements were done when steady state conditions were present.

The results are given as per cent uncovered area and presented as columns in Fig 7. Thus a high column indicates a low tendency to retain air bubbles.

Flow capacity could be tested both in the equipment shown in Fig. 1 and in the equipment shown in Fig. 2. The results were closely comparable. Each pattern was tested twice with a constant negative transmembrane pressure at the outlet (TMP=150 mmHg) in all experiments. The pressure difference (ΔP) between dialysate inlet and outlet was measured at dialysate flows of 50, 100, 150, 200, 250 and 300 ml/min. The values for each pattern are given as the flow (ml/min) at ΔP of 5 and 20 mmHg and are presented as the flow capacity in Fig. 7. A high column thus represents a low resistance.

Membrane compliance was tested in a pressure cell shown in Fig. 3. One chip of the membrane support pattern is fitted in the base of the sealing rim. The cell is mounted under water to prevent air retention within the cell. A manometer filled with silicone oil (to reduce the elasticity of the volume) and a syringe containing water is connected through the lid. The deflexion of the membrane is determined by measuring the increase in pressure as

the contents of liquid in the syringe is displaced into the cell. Repeated trials with the same membrane gives different graphs because a permanent stretching of the membrane occurs. A new membrane must therefore be fitted for each trial. The elasticity of the system is determined in the same manner by using chips with a smooth surface made in the same way as the chips with patterns. The true membrane elasticity on the tested support pattern is thus the difference between these two measurements. Six test series were carried out with the membrane oriented in two perpendicular directions in each test. The membrane is anisotropic and expands approximately 10% when moistened in its longitudinal axis but only of a minor order in its transverse axis. The values did not deviate much but the presented values are those with the lateral axis of the membrane parallel to the direction of the dialysate flow (as this is the way it would be oriented in the parallel-plate dialyser).

From the resulting almost linear graphs the slope is determined. The values were found in every given support to vary less than $\pm 3\%$ and the average value is given as the pressure increase as a function of volume increase (ATO per ml). A high value presented as a high column in Fig. 7 thus represents a support with stiff bracing properties.

Membrane rupture value was tested in the same

RESULTS

27 different injection moulded types of membrane supports were analyzed. 17 of the supports were however found to have so faulty properties in one or more tests that they were rejected before comparative evaluation. The remaining 15 were compared as shown in Table I. This table gives in numbers the results of the tests. In Fig. 7 the values are shown as columns. A high column indicates good performance of each property. The support which combined good merits for all the tested parameters was selected for use in the dialyser to be constructed.

From the experimental results efficiency graphs for finer evaluation were computer drawn by an IBM 1130 with a Plotter 1442 for all the supports.

The least square fitted curve for the dialysis time was calculated and the resulting fitted graph for the permeation factor P was calculated. At the highest value of P the ideal graph for the theoretically best dialysis time was calculated. Figs. 4-6 give three examples of typical graphs. The graph with triangular values is P the graph with superimposed standard deviations is the experimental performance. The broken graph represents the "ideal" graph (with constant P of the support when it is best).

In Fig. 4 P increases as Q increases, indicating a progressive improvement of the dialysate mixing, probably due to a reduction of the unstirred layer phenomenon.

In Fig. 5 P rises from one level to higher level probably partly as a result of the release of airbubbles in the support pattern when Q increases.

In Fig. 6 P maintains an almost constant value throughout the whole flow range indicating a uniform all-round performance.

DISCUSSION

Although the obtained results give a firm basis for the selection of a membrane support with uniform performance within a wide range of dialysate flow values, it should be pointed out that the performance of a dialyser with the support in question cannot fully be predicted because the dimensions of the individual blood layers as well as the dialyser as a whole also has profound influence. From Table I which gives the summary of the values on the best support patterns one can see that a higher permeation factor is obtained with



Fig. 8 A cast of the membrane resting on support no. 13 at 500 mmHg. Note the uniform setting of the membrane.

cell in connection with the compliance measurements. The rupture value was recorded as the pressure when the membrane gave in. The values did not vary more than 5% and the mean value is given in 180 m in Fig. 7. Thus a high value represents a support with good bracing properties.

Topography. *f* blood layer was evaluated at TMP of approximately 400 mmHg by making a cast with a suspension of dental plaster of Paris which does not shrink during setting. Each cast was studied to learn how the membrane settled on the support pattern. A poor adjustment of the membrane resulted in sharp bends in the profile. We judged it important to obtain a very even blood contact surface in bends and wrinkles were considered potential sites for thrombus formation. The results are not shown but they were considered during the selection of the best support.

Fig. 8 shows an example of a uniform settlement of the membrane over an area of membrane support.



Fig. 7 Diagrammatic representation of the evaluated properties of 15 membrane supports. High columns indicate good performance in the particular parameter. The differences

between the best patterns are marginal. Pattern no. 13 was chosen for support in the dialyser.

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the contents of liquid in the syringe is displaced into the cell. Repeated trials with the same membrane gives different graphs because a permanent stretching of the membrane occurs. A new membrane must therefore be fitted for each trial. The elasticity of the system is determined in the same manner by using chips with a smooth surface made in the same way as the chips with patterns. The true membrane elasticity on the tested support pattern is thus the difference between these two measurements. Six test series were carried out with the membrane oriented in two perpendicular directions in each test. The membrane is anisotropic and expands approximately 10% when moistened in its longitudinal axis but only of a minor order in its transverse axis. The values did not deviate much but the presented values are those with the lateral axis of the membrane parallel to the direction of the dialysate flow (as this is the way it would be oriented in the parallel-plate dialyser).

From the resulting almost linear graphs the slope is determined. The values were found in every given support to vary less than $\pm 3\%$ and the average value is given as the pressure increase as a function of volume increase (ATQ per ml). A high value presented as a high column in Fig. 7 thus represents a support with stiff bracing properties.

Membrane rupture value was tested in the same

7	8	9	10	11	12	13	14	15
Z-pattern, fine	Cross- pattern	U-pattern	Cross- pattern	Cross + Tenon	Cross- ridge, fine	Cross- ridge, medium	Cross- ridge, course	Cross- ridge large
24.8 (5.3)	34.1 (1.3)	42.0 (3.2)	17.7 (9.9)	40.4 (4.1)	39.7 (4.7)	42.5 (0.8)	43.1 (1.4)	41.9 (5.2)
30.6 (0.7)	37.0 (2.3)	43.3 (3.5)	42.1 (2.0)	43.4 (2.2)	44.7 (1.5)	44.2 (1.7)	30.7 (2.3)	44.1 (2.6)
5.6 (0.1)	7.4 (0.1)	3.6 (0.0)	5.6 (0.1)	7.1 (0.1)	7.3 (0.1)	5.7 (0.1)	4.3 (0.1)	3.7 (0.1)
14 (1.3)	11 (1.4)	19 (1.2)	30 (1.9)	24 (1.1)	36 (1.3)	26 (1.3)	20 (1.2)	19 (1.2)
56 (1.3)	30 (1.4)	83 (1.2)	218 (1.8)	109 (1.2)	135 (1.3)	123 (1.2)	91 (1.3)	88 (1.2)
5.3 (0.0)	3.4 (0.1)	3.9 (0.0)	2.3 (0.1)	1.9 (0.1)	6.7 (0.1)	3.6 (0.1)	2.9 (0.0)	1.9 (0.1)
9	8	5	13	7	5	1	3	2

the clinician because it could result in a high extra-corporeal volume at high transmembrane pressure gradients. Furthermore the sagging results in an increased shading of the membrane. By introducing a thicker and stronger membrane this tendency can be reduced but this often means an increased diffusion resistance. The membrane used in these experiments is—as earlier mentioned—anisotropic and packing the membrane (as performed in production of dialysers) in a dry condition can result in an uneven bracing when it becomes wet.

For this reason a membrane support with parallel oriented ridges braces anisotropic membranes in a more uniform fashion than a support pattern where the ridges form angles or are oriented in more than one direction. This could be shown on the casts made of the membrane topography. It is of interest to note that although a good compliance of a support is achieved this does not necessarily go for the rupture level. A low rupture level value must indicate that a localized concentrated drag is applied on the membrane and the plaster casts indicate that

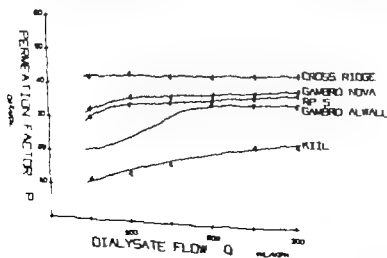


Fig. 10 The graph shows the permeation factor for patterns from 4 well known dialysers in clinical use. The graph denotes cross ridge represents support no. 13.

Table I

Membrane support no	Description of support patterns							
	Unit	n	Pyramid (1.2 mm)	Pyramid (0.8 mm)	Semilunar coarse	Semilunar + Tenon	Semilunar fine	Z-pattern coarse
Permeation factor at 50 ml/min	cm ³ /min	24	20.5 (0.7)	28.0 (2.6)	37.8 (2.5)	33.1 (1.4)	44.5 (4.2)	23.7 (4.2)
Permeation factor at 300 ml/min	cm ³ /min	24	38.5 (0.7)	50.5 (6.8)	52.2 (1.9)	45.3 (1.0)	51.3 (2.8)	30.0 (4.1)
Compliance	ATO/ml	6	2.0 (0.1)	2.0 (0.1)	.9 (0.1)	4.4 (0.0)	3.5 (0.0)	5.2 (0.1)
Flow capacity at 5 mmHg	ml/min	12	62 (1.2)	55 (1.3)	39 (0.9)	71 (1.2)	27 (1.4)	33 (1.1)
Flow capacity at 70 mmHg	ml/min	12	272 (3.1)	741 (2.8)	167 (1.4)	92 (1.2)	124 (1.2)	143 (1.8)
Rupture level	ATO	6	2.1 (0.1)	2.1 (0.1)	5.5 (0.1)	1.3 (0.0)	6.1 (0.1)	2.3 (0.0)
Air retention at 50 ml/min	% of area	1	7	3	6	10	5	9

most of the supports with increasing dialysate flow. This is in our opinion an important fact which has frequently been overlooked. A low velocity in the dialysate will in most supports tend to formation of unstirred boundary layers and—more important—retention of air bubbles which normally are abundantly present as a result of insufficient

de-aeration of the dialysate. Both of these disadvantages can be reduced or eliminated if recirculation of dialysate is used—a method that consumes no more dialysate. In this case the flow capacity of the support pattern becomes a most important factor.

Compliance is normally considered important by

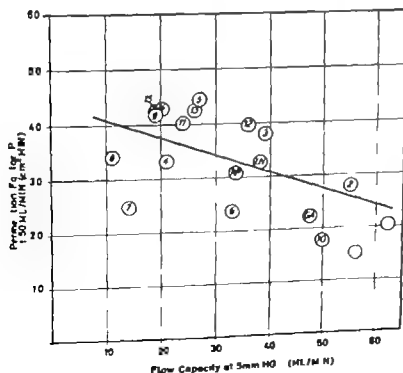


Fig. 8 The permeation factor at 50 ml/min plotted against the flow capacity at 5 mmHg for the tested support patterns. The figure in the circles indicate the pattern number. The values of Gambro Alwall (GA), Gambro Lundia Nova (LN), Rhone Poulenc's RP 5 (RP) and Aml (A) are inserted. The graph represents the least square-fitted graph.

$$(P = 44.3953 - 0.333 Q) \quad r^2 = 0.3136$$

A New Parallel Plate Dialyser

II. Description of Design and In Vitro Performance

S. Dawids and C. Boe

From Medical Department P, Division of Nephrology, University Hospital, Rigshospitalet, Copenhagen and Department of Mechanical Engineering, Technical University of Denmark, Lyngby, Denmark

ABSTRACT Using a high efficiency membrane support pattern described in the previous paper, a prototype of a new parallel plate dialyser has been developed. The new dialyser is designed in such a way that the number of components is reduced to one item per blood layer and the membrane is folded as a continuous. This permits a semiautomatic assembly of the unit, which reduces production costs. Furthermore this technique facilitates aseptic handling of the Mem. The in vitro performance has been tested and it compares favourably with commercially available disposable parallel plate dialysers.

INTRODUCTION

The clinical applicability of a dialyser depends primarily on its efficiency in extracting uremic waste products and removing water through ultrafiltration. However a number of other requirements are necessary before the dialyser is accepted in the clinic and these can be roughly summarized as follows.

1. Non-toxic sterilizable materials of uniform quality must be used combined with good blood compatibility.

Extraction ability (i.e. clearance) must be good and reproducible even during high transmembrane pressure.

2. Good controllable, reproducible ultrafiltration.

3. Low frequency of membrane rupture.

4. A low blood volume and good compliance combined with low flow resistance to blood and dialysate.

5. Low amount of residual blood at termination of treatment.

6. Pleasant appearance and easiness of handling combined with good storage properties.

8. Low priced (indicating simple and rapid manufacturing).

As some of these points are somewhat conflicting the resulting dialyser design usually will have to emphasize a few of the above mentioned requirements at the expense of other less important virtues.

Although no exact knowledge exists as to the nature of the uremic toxins the suggestion that medium range molecules may play a significant role in the uremic syndrome (2, 3, 4, 12, 13, 22, 23, 26) has spurred research on new materials for membranes (5, 9, 10, 11, 14, 15, 17, 19, 20, 24). Cellophane has however so far proven superior primarily because of its uniform quality and its price. Thin types of cellophane show an increased permeability to middle molecules as compared with thicker membranes (9, 14, 16, 25, 27). Unfortunately this advantage of the thin membrane is counterbalanced by its weaker structure resulting in a higher tendency of rupture and thus underlines the necessity of a careful and individualized design of the membrane support in the dialyser (1, 6, 8, 13, 28).

GENERAL DESCRIPTION

The tested prototype (Fig. 1) contains 14 parallel blood layers each formed between simple plates inserted in pouches of the membrane.

The single injection moulded plate (Fig. 2) includes the ports for blood and for dialysate as integrated parts. The blood ports are folded into a spindle shaped congruent concavity on the plate allowing a very smooth transition between the surfaces of the blood port and those of the plate. The formation of very fine flash lines at the transition points reduce possible leaks at that critical location to a negligible size.

this is on corners points and shoulders of ridges especially if these are only slightly rounded. Off hand one would expect that an efficient mixing resulted in a higher flow resistance (equal to a reduced flow capacity). A plot of permeation factors at 50 ml/min versus flow capacity shown on Fig. 9 confirms this. The considerable deviation from this rule may be caused by variable air bubble retention and shading in the low range of the testing conditions i.e. at dialysate flows of 30–100 ml/min. (which corresponds to the flow velocity of dialysate in clinical dialysis).

On the basis of the results shown in Table I support no. 13 was chosen for the dialyser design. The best supports have admittedly only marginal differences in the properties and the decision was based on the overall performance with emphasis on the clinically important parameters (efficiency, compliance, rupture level and air retention).

The support constructed on the basis of our tests compares favourably with support patterns of 4 commercially available dialysers (Fig. 10). It should however again be emphasized that these results per se do not reflect the performance of the respective dialysers which does not only depend on the support pattern.

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A New Parallel Plate Dialyser

II. Description of Design and *In Vitro* Performance

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*From Medical Department P, Division of Nephrology, University Hospital, Rigshospitalet
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ABSTRACT Using a high efficiency membrane support pattern, described in the previous paper, a prototype of a new parallel plate dialyser has been developed. The new dialyser is designed in such a way that the number of components is reduced to one liter per blood layer and the membrane is folded as a continuous. This permits a semiautomatic assembly of the unit, which reduces production costs. Furthermore this technique facilitates aseptic handling of the item. The *in vitro* performance has been tested and it compares favorably with commercially available disposable parallel plate dialysers.

INTRODUCTION

The clinical applicability of a dialyser depends primarily on its efficiency in extracting uremic waste products and removing water through ultrafiltration. However a number of other requirements are necessary before the dialyser is accepted in the clinic, and these can be roughly summarized as follows:

1. Non-toxic sterilizable materials of uniform quality must be used combined with good blood compatibility.

2. Extraction ability (clearance) must be good and reproducible even during high transmembrane pressure.

3. Good controllable reproducible ultrafiltration.

4. Low frequency of membrane rupture.

5. A low blood volume and a good compliance combined with low flow resistance to blood and dialysate.

6. Low amount of residual blood at termination of treatment.

7. Pleasant appearance and easiness of handling combined with good storage properties.

8. Low priced (indicating simple and rapid manufacturing).

As some of these points are somewhat conflicting the resulting dialyser design usually will have to emphasize a few of the above mentioned requirements at the expense of other less important ones.

Although no exact knowledge exists as to the nature of the uremic toxins the suggestion that medium range molecules may play a significant role in the uremic syndrome (2, 3, 4, 11, 13, 22, 33, 36) has spurred research on new materials for membranes (5, 9, 10, 11, 14, 15, 17, 19, 20, 24). Cellophane has however so far proven superior primarily because of its uniform quality and its price. Thin types of cellophane show an increased permeability to middle molecules as compared with thicker membranes (9, 14, 18, 33, 37). Unfortunately this advantage of the thin membrane is counterbalanced by its weaker structure resulting in a higher tendency of rupture and thus underlines the necessity of a careful and individualized design of the membrane support in the dialyser (1, 6, 8, 21, 23, 28).

GENERAL DESCRIPTION

The tested prototype (Fig. 1) contains 14 parallel blood layers each formed between sample plates inserted in pouches of the membrane.

The single injection moulded plate (Fig. 2) includes the ports for blood and for dialysate as integrated parts. The blood ports are folded into a spindle shaped congruent concavity on the plate allowing a very smooth transition between the surfaces of the blood port and those of the plate. The formation of very fine flash lines in the transition points reduce possible leaks at this critical location to a negligible size.

this is on corners points and shoulders of ridges especially if these are only slightly rounded. Off hand one would expect that an efficient mixing resulted in a higher flow resistance (equal to a reduced flow capacity). A plot of permeation factors at 50 ml/min versus flow capacity shown on Fig. 9 confirms this. The considerable deviation from this rule may be caused by variable air bubble retention and shading in the low range of the testing conditions i.e. at dialysate flows of 30–100 ml/min (which corresponds to the flow velocity of dialysate in clinical dialysis).

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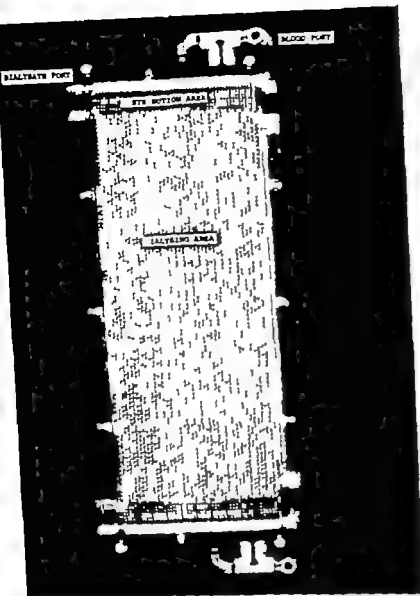


Fig 2 A single injection moulded plate. Note the spindle shaped variation in thickness of the blood port and the corresponding sealing areas.

factors have no injurious influence on the dialysers efficiency. They do however play a role during the wash-out of blood with saline at the termination of dialysis. The volume of saline required will be approximately 30 per cent greater than if the multiple flow paths were of exactly identical length.

DIALYSER IN VITRO TESTING

Prior to the regular testing it was observed that slight variation occurred in the properties of the membrane from batch to batch. Similar variations

are well known (14, 15, 16, 26) and can amount to 10% in ultrafiltration rate and 5-6% in clearance. Consequently each test in the subsequent series was run on dialysers built with membrane material from a chosen batch with average properties.

Priming volume and compliance was measured in 6 dialysers from the same batch. The blood compartment was filled with liquid while the dialyser was suspended from a balance (Sartorius type 2E) mounted with a displacement transducer (Kroijer Jensen type LDI 9050) in connection with a carrier wave bridge (K J type 5100 A). The static pressure

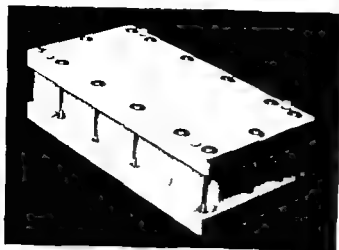


Fig 1a Prototype A of the Boe-Dawids dialyser

As the dialyser is built the membrane is folded back and forth as a continuum every other time inserting a plate from one side into the formed pouches and subsequently folding the blood ports into the pouches opening towards the opposite side. The correct mutual positioning of plates and blood ports is obtained by taps along the edges which fit into corresponding vertical holes on the next plate. The vertical blood distribution channels are formed by the stacked blood ports and the dialysate distribution is in principle the same with a vertical channel formed by the dialysate ports. The blood layers are sealed by sealing rims along the borders of the plates and blood ports.

The principle of this design not only ensures a simplified production because only one moulding form is necessary but also ensures a good tolerance fit between the plate and the blood ports in spite of variable shrinking properties of the plastic material. The design results in an effective blood-dialysate separation because the blood and dialysate enter each side of the dialysing area over a folded part of the membrane without any free edges at the entry sites. If a minor leak e.g. at the dialysate ports should occur this can only drain to the exterior of the dialyser with no chance of entering the blood compartment. Membrane leakage of fluids only occurs when there is actual rupture of or hole in the membrane.

INTERNAL FLOW PATTERN

The flow pattern in the blood layer is influenced by the fact that entry and exit of the blood is located along the same side of each plate with a shallow

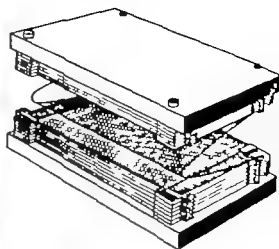


Fig 1b Diagram showing the mutual positioning of injection moulded plates, blood ports and the folded membrane

relatively narrow blood distribution area extending across the surface to the opposite border of the plate. Blood entering the membrane area via the blood port is spread out in the blood distribution area and subsequently directed over the dialysing area. The blood which flows near the edge where the blood ports are located will enter the dialysing area immediately after it has entered the blood distribution area and earlier than the blood which travels varying lengths in the blood distribution area. Thus the entering blood will form an oblique advancing front across the dialysing area. At the exit an additional delay occurs because of the symmetry of the plate.

Accordingly experiments with contrast injected selectively as a bolus into individual blood layers in a dialyser during X-ray screening revealed an oblique contrast front. This front moved evenly along the dialysing area indicating that flow distribution across the single blood layer was uniform.

The blood layers are located at different distances from the dialyser's blood entry and exit. As a result the blood reaches the upper blood layers before it reaches the bottom layers and again the blood from the top layers reaches the exit relatively earlier because of the symmetry.

Contrast injection revealed a slightly higher flow in the bottom layers, however, not of a magnitude to measurably reduce the delay discussed above.

Since the velocity in the blood compartments is practically identical within layers as well as between layers the above mentioned design related

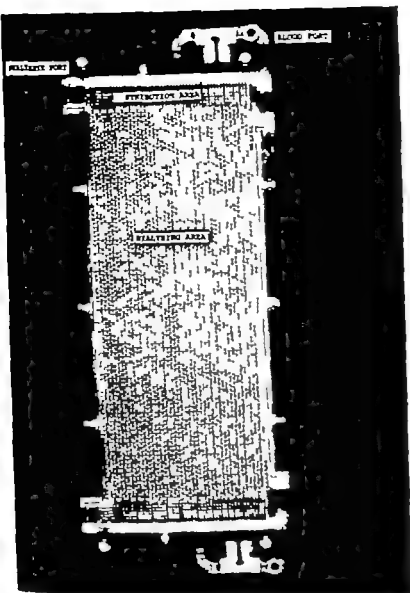


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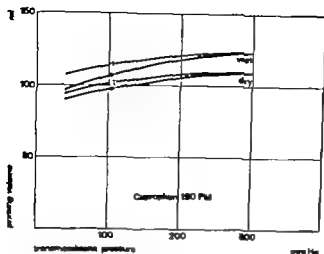


Fig 3 Priming volume as a function of transmembrane pressure in dry (silicone oil-filled) and wet (water-filled) in the prototype. Stretching of the membrane during high TMP results in a subsequent permanent increase of volume

in the blood compartment was measured with an integrated transducer unit (Tyco Model AB 0-15 psi)

A simultaneous calibrated recording of the weight (i.e. volume) as a function of the transmembrane pressure was recorded on an X Y recorder (Moseley Autograf Model 7000 A R). Each test was run within 30 seconds to avoid errors from ultrafiltration. The pressure was increased from 0 to 300 mmHg. The weight was measured better than 0.5 g and the transmembrane pressure (TMP) was measured better than 1 mmHg. Tests were made on 3 dry (silicone oil filled) and 3 wet (water filled) dialysers

The results given in Fig 3 show a priming volume of 91 ml in the dry condition and 97 ml when the membrane is wet. It is seen that with increasing pressure on the membrane a stretching of the film occurs resulting in an increase of blood volume. At 300 mmHg the volume is 110 ml and 124 ml in dry and wet conditions respectively. When the pressure is relieved the shrinking is incomplete (dry 95 ml and wet 107 ml) indicating that a permanent stretching of the membrane has taken place.

Flow resistance in the blood compartment—defined as the pressure difference in mmHg between entry and exit of the blood compartment—was made on 4 dialysers. A water flow of $200 (\pm 3)$ ml/min at 20°C was established with an occluding laboratory rollerpump. The flow was read on a flow meter at the exit (Brook D & R 6-15 A)

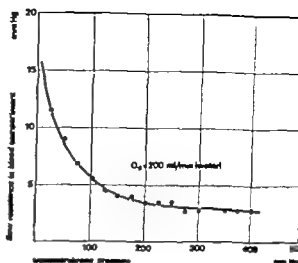


Fig 4 Flow resistance in the blood compartment at 200 ml/min as a function of transmembrane pressure

The dialysate compartment was left open. The transmembrane pressure defined as the mean value in the blood compartment and read with precision manometers (NAF aneroid) at entry and exit was initially set at a value of approx. 25 mmHg and subsequently increased in steps of 25 mmHg up to TMP of 400 mmHg. Sixteen sequential measurements were made in the range 25-400 mmHg.

The results given in Fig 4 show that the pressure drop through the blood compartment at clinical levels of transmembrane pressure (TMP) (i.e. 100-200 mmHg) is approximately 5 mmHg at a flow in the blood compartment of 200 ml/min.

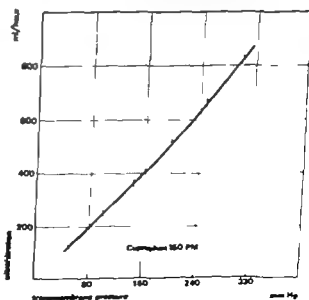


Fig 5 In vitro ultrafiltration rate (ml/hour) as a function of transmembrane pressure

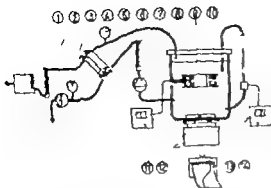


Fig. 6 Schematic diagram of the testbench for clearance measurements. (1) Through-flow thermostat, (2) Dialysate flow meter with flow regulator (3) Effluent pump (4) Manometer showing the relative dialysate pressure level, (5) Manometer showing the bulk pressure in the dialyser's blood compartment, (6) Dialyser being tested (7) flowmeter showing the entry flow in the blood compartment, (8) laboratory roller pump (9) reservoir with solute, (10) small laboratory roller pump circulating solute (11) inductive conductivity sensor (12) laboratory heater with magnetic stirrer (13) recorder (14) concentration measurement of organic solutes based on ultraviolet absorption

Ultrafiltration was measured as the reduction of volume of 2 liters of saline recirculated through the blood compartment. A constant dialysate flow of $600 \text{ ml/min} \pm 10 \text{ ml/min}$ through the dialysate compartment was maintained throughout the testing period. The transmembrane pressure was established with an effluent pump earlier described (7).

The ultrafiltered volume was recorded in 3 subsequent periods of 20 minutes at 3 TMP values (50, 100, 144, 199, 249 and 300 mmHg). The average values shown in Fig. 5 are given in ml/hour. Variations were found to be within 6% provided dialysers used for testing were mounted with membrane material from the same batch.

The results shown in Fig. 5 show an almost linear correlation between the transmembrane pressure and ultrafiltration rate with a mean value of 252 ml/h at TMP of 100 mmHg and 821 ml/h at 300 mmHg.

Membrane rupture resistance defined as the TMP value at which the membrane in the dialyser ruptures was tested on six dialysers on a simple test bench.

A water reservoir containing 0.5 liter solution of dark brown 0.1% dextran-iron complex ($M=75,000$, Infonorm, 0.1% dextran-iron complex) was used

of the dialyser and to a valve from which water is replenished during the violent ultrafiltration at high TMP values. The dialysate compartment is left open. A small pneumatic oscillator exposes the blood compartment of the dialyser via the reservoir to an intermittent pressure of 5 seconds duration and a subsequent period of 5 seconds with no pressure. Each dialyser was exposed to a stepwise increasing pressure every two hours until a TMP maximum of 1900 mmHg (2.5 Atm) was reached after 10 hours and exposure at this level was maintained at least for 120 hours.

The TMP levels at which rupture of the membrane occurred was not determined as none of the six tested dialysers showed any sign of membrane ruptures, detected as brown discoloration of the ultrafiltrate. At even higher pressure levels the dialysers clamping system gradually gave in and caused progressive leaking to the exterior of the dialyser.

Clearance was tested for chloride ($M=35.5$) creatinine ($m=113$) uric acid ($M=168$) bromsulphalein ($M=838$) polyethylene glycol ($M=1000$) and B_{12} ($M=1355$).

The clearances were determined on a testbench shown schematically in Fig. 6. A reservoir (9) containing 5 liters of aqueous solution is stirred and thermostated to 38°C (12) with a combined magnetic stirrer and heater (Hedolph type MK II). The solution is continuously recirculated with an adjustable laboratory roller pump (8) via a flowmeter (Brook D&R 6-15A) (7) through the blood compartment of the dialyser (6) and returned to the reservoir. Tap water thermostated to 38°C in a laboratory through-flow thermostat (1), is used as dialysate and flows counter-current via another flowmeter (F&P Co. 3F 3/8) (2) through the dialysate compartment. A negative dialysate pressure of 90 mmHg is established with an effluent pump (3) described in an earlier paper (7) and adjusted according to readings on a precision manometer (NAF) (4). The pressure at the outlet of the blood compartment is held constant at 130 mmHg with an overflow arrangement on the return line to the reservoir. The concentration of chloride is measured with an inductive conductivity meter (11) also previously described (7). The time related chloride measurement is recorded (Servograph recorder type RE 511) (13). Organic solutes (creatinine, uric acid and bromsulphalein) are separately measured in

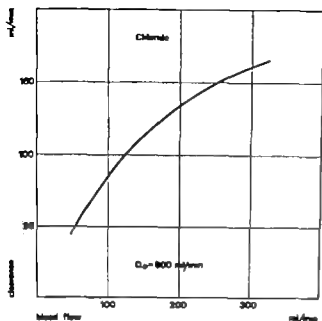


Fig 7 In vitro clearance of chloride as a function of variable Q_D

and likewise recorded (13). The time related concentrations are subsequently derived from standard graphs. An exception from this type of measurement was the B_{12} test where isotope tagged B_{12} was used. The vitamin solution containing $10 \mu\text{Ci CO}^{58}$ was mixed with 10 liters of water which was recirculated through the dialyser. Five ml samples were drawn at each run. Six tests were made at different blood flow values (50 100 150 200 250 300 ml/min). The activity was determined in a liquid scintillation counter (Hewlett Packard Armarc Model 446) with an error less than 0.5%. The efficiency Cl (clearance) was calculated from the equation

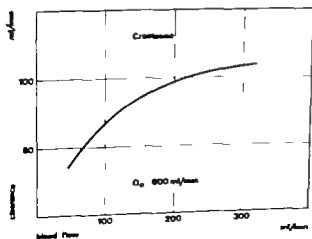


Fig 8 In vitro clearance of creatinine as a function of variable Q_D

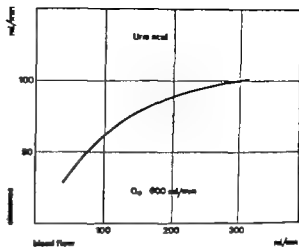


Fig 9 In vitro clearance of uric acid as a function of variable Q_D

$$Cl = \frac{\text{Vol} \ln(C_0/C_1)}{t} \text{ (ml/min)}$$

where Vol is the average volume during the test C and C_0 are concentrations initially and at the time t .

The results are given in Figs 7-12. At blood flow of 200 ml/min and dialysate flow of 600 ml/min the in vitro clearance values were the following: Chloride 139 ml/min, creatinine 96 ml/min, uric acid 87 ml/min, bromsulphalein 37 ml/min, polyethylene glycol (Carbowax® 1000) 35 ml/min, B_{12} 44 ml/min.

With a dialysate flow of 1000 ml/min and unchanged blood flow the values increased as follows: Chloride 141 ml/min, creatinine 101 ml/min, uric acid 89 ml/min, bromsulphalein 37 ml/min, PEG (1000) 35 ml/min, B_{12} 45 ml/min.

The relation between clearance and varying dialysate flow at constant blood flow is given in Fig 13 for chloride and creatinine. It is seen that an increase of dialysate flow from 600 to 2000 ml/min

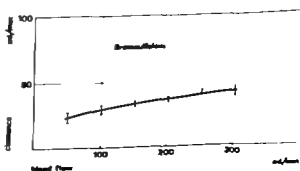


Fig 10 In vitro clearance of bromsulphalein as a function of variable Q_D

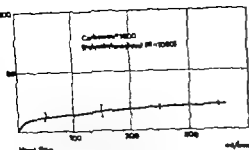


Fig. 11 In vitro clearance of polyethylene glycol ($V=1000$) as function of variable Q_D .

only results in a moderate gain of clearance being 5% for chloride and 3% for creatinine. It should be noted that a significant increase of efficiency could not be seen when a local recirculation (2000 ml/min) was established (not shown in figure).

DISCUSSION

The chief merit of this dialyser is considered to be the simple design which is achieved by integrating the blood and dialyser ports, sealing rims etc. in the plate and by using one single length of membrane for each dialyser thereby avoiding manufacture and manipulation of separate membrane sheets for each layer. The semiautomatic assembly enables a cheaper production. Furthermore it ensures larger safety against contamination since a no-touch technique can be employed which is of special importance in the handling of the membrane and the blood compartment. The experimental results indicate that the clinically important values of a dialyser are contained in this prototype.

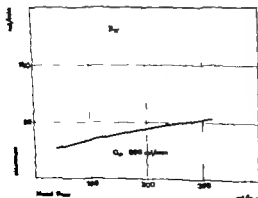


Fig. 12 In vitro clearance of vitamin B as function of variable Q_D .

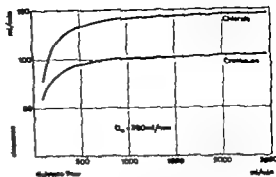


Fig. 13 In vitro clearance of chloride and creatinine as function of variable dialysate flow Q_D .

The efficiencies (e.g. creatinine clearance of 96 ml/min at blood flow of 200 ml/min and dialysate flow of 600 ml/min) are very satisfactory in particular as these results are obtained with a dialysing area of only 0.94 m². An increase of area to 1 m² would increase the estimated efficiency approximately by 5-6% taking into account the resulting lower flow velocities in the individual layers.

The membrane support pattern thus seems to live up to the expectations of a relatively high efficiency at low dialysate flow velocities. The remarkably good clearance with polyethylene glycol and B₁₂ indicates a good efficiency in the range of middle molecules. The tests with variable dialysate flow (Fig. 13) show that dialysate flow above 550-600 ml/min in this dialyser only adds a little to the efficiency whereas a reduction of the dialysate flow below 500 ml/min progressively will reduce the extraction.

The above mentioned underlines that a sufficient blood flow through a dialyser is the main prerequisite for a good clearance.

The in vitro ultrafiltration shows modest values again because of the small dialysing membrane area in the prototype (0.94 m²). Corrected values for a membrane area of 1 m² would be approximately 310 ml/h per m² at TMP of 100 mmHg.

Table I Technical specifications on Bor-Davids dialyser (prototype)

Weight without clamping	1.5 kg
Weight with clamping	2 kg
Blood layer	14
Membrane area	0.94 m ²
Dimensions: 33 × 11 cm.	

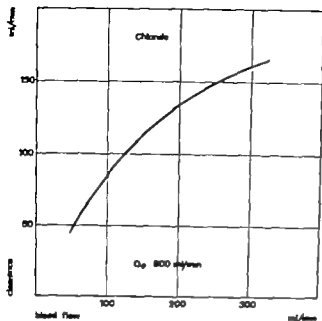


Fig 7 In vitro clearance of chlorid as a function of variable Q_p

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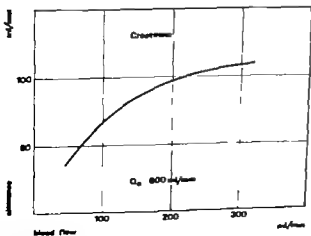


Fig 8 In vitro clearance of creatinine as a function of variable Q_p

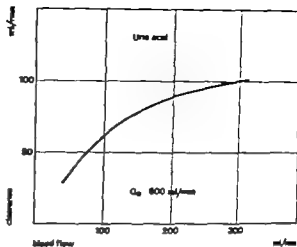


Fig 9 In vitro clearance of uric acid as a function of variable Q_p

$$Cl = \frac{\text{Vol} \ln(C_i/C_t)}{t} \text{ (ml/min)}$$

where Vol is the average volume during the test. C_i and C_t are concentrations initially and at the time t .

The results are given in Figs 7-12. At blood flow of 700 ml/min and dialysate flow of 600 ml/min the in vitro clearance values were the following: Chloride 139 ml/min, creatinine 96 ml/min, uric acid 87 ml/min, bromsulphalein 37 ml/min, polyethylene glycol (Carbowax® 1000) 35 ml/min, B_{12} 44 ml/min.

With a dialysate flow of 1000 ml/min and unchanged blood flow the values increased as follows: Chloride 141 ml/min, creatinine 101 ml/min, uric acid 89 ml/min, bromsulphalein 37 ml/min, PEG (1000) 35 ml/min, B_{12} 45 ml/min.

The relation between clearance and varying dialysate flow at constant blood flow is given in Fig 13 for chloride and creatinine. It is seen that an increase of dialysate flow from 600 to 7000 ml/min

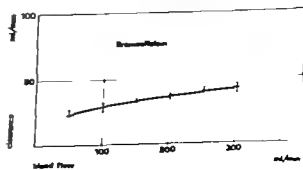


Fig 10 In vitro clearance of bromsulphalein as a function of variable Q_p

A New Parallel Plate Dialyser

III Preliminary Experience on Clinical Performance

S. Dawids and S. Vojnovic

From Department F, Division of Nephrology, University Hospital, Rigshospitalet, Copenhagen, Denmark

ABSTRACT The clinical model of a new parallel plate dialyser with a membrane area of 0.96 m^2 with copolyester (SP PA) has been tested in more than 2000 dialyses with good clinical results. The simple design permits an aseptic, semiautomatic assembly and makes cheap industrial production possible. Clinical clearance values for urea, creatinine and uric acid are presented as well as data for ultrafiltration, residual blood volume and rupture frequency. The specific efficiency per square meter has been found to be high.

With the advent of large-scale haemodialysis treatment the need for low priced disposable equipment has become urgent. A model of a semiautomatically manufactured parallel plate dialyser has been tested in more than 2000 dialyses with good biochemical and clinical results.

The design and construction of the dialyser has been described earlier (2). This paper reports on our preliminary experience with a clinical model shown in Fig. 1 with a dialysing area of 0.96 m^2 and sterilized by irradiation (minimum dose 3... megarad).

(1) *Residual blood volume* in the dialyser was determined randomly in 52 oxine dialyses at the termination of the treatment. The dialysers were taken apart and the membrane was thoroughly washed together with the blood ports to 1 liter of saline. The mixture was haemolysed with 2 drops of Triton X⁸ and the absorption of filtered sample was measured spectrophotometrically at 540 μ (Beckmann Model B 17000). From the patient blood sample was taken from the venous blood line prior to the termination of dialysis. The sample was likewise haemolysed and diluted with saline to 1/125, 1/250, 1/500 and 1/1000 corresponding to 2, 4 and 1 ml blood in 1 liter respectively. From the resulting graph the quantity of blood in the washing water could be determined within 0.2 ml.

The mean residual blood loss was found to be

3.1 ml (range 0.3-6.5 S.D. = 1.3). As the erythrocytes trigger the coagulation cascade (1, 4, 7) through release of ADP it has been thought that higher haematocrit values could lead to higher clot formation with resultant larger blood loss. As shown in Fig. 2 no significant correlation to the patient's haematocrit (correlation rate = 0.007) could however be detected in the present tests.

The ultrafiltration rate was recorded during a 2 hours period of dialysis with a blood flow above 150 ml/min, and was estimated from the weight reduction which was measured on a bed balance (Dalex, WJ 104) better than $\pm 10 \text{ g}$. Transmembrane pressure (TMP) was recorded via a transducer previously described (3) and was held constant during the two hours. Measurements of the relationship between TMP and ultrafiltration rate were made during 205 dialyses.

In Fig. 3 the ultrafiltration rate in ml per hour is given as a function of the transmembrane pressure. The dispersion is shown as a shaded area around the mean graph which shows an ultrafiltration rate of approx. 300 ml/h at a TMP of 200 mmHg and approx. 1025 ml/h at a TMP of 400 mmHg.

(b) *Clearance* was calculated according to the conventional equation.

$$Cl = Q_b \cdot E \quad (1)$$

and

$$E = \frac{C_m - C_{m_0}}{C_m} \quad (2)$$

where Cl = clearance, Q_b = blood flow, E = extraction, C_m = blood concentration at entry and C_{m_0} = blood concentration at outlet.

In all cases an occlusive roller pump (Travenol) was used. The blood flow was determined indirectly by measuring the time required for 25 revolutions of the roller pump head. By relating rotation speed to a volumetric determination before and after

A New Parallel Plate Dialyser

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■ Dawids and S. Vojnovic

From Department P Division of Nephrology University Hospital, Rigshospitalet Copenhagen, Denmark

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where Cl = clearance, Q_b = blood flow, E = extraction, C_{in} = blood concentration at entry and C_{out} = blood concentration at outlet.

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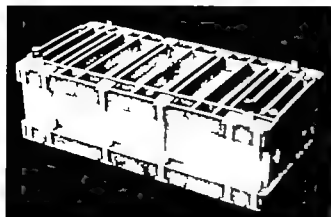


Fig 1 The prototype B of the Boe-Dawids dialyser

ter the dialysis the error was found to be within $\pm 2.5\%$. Blood samples were drawn simultaneously from the arterial and venous lines in 5 ml syringes. These were held in an upright position for approximately 15 minutes to allow sedimentation of the erythrocytes which thereupon were returned to the blood circulation. Analyses were made for urea, creatinine and uric acid in the plasma.

The results are given in Figs 4-6. Clearance values are at a bloodflow of 200 ml/min: urea=122 ml/min, creatinine=96 ml/min and uric acid=81 ml/min. These clearances have been measured both with single pass of 600 ml/min shown as black spots and with local recirculation of 7500 ml/min indicated by open spots. It is seen that increased velocity of the dialysate results in an insignificant increase of the efficiency.

Rupture frequency. Blood leaks were detected in barely 2% of all dialysers. 2/3 of these were faint

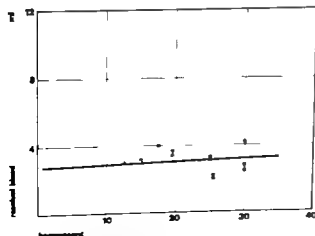


Fig 2 Residual blood volume as a function of haematocrit value. No correlation to the haematocrit is present.

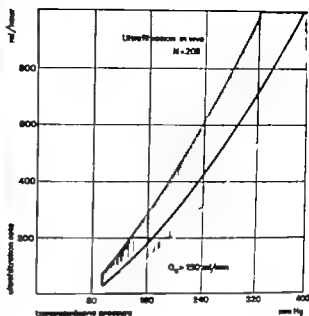


Fig 3 In vivo ultrafiltration rate as a function of TMP. The shaded area covers the 96% confidence limits.

mostly transient leaks barely visible in the effluent dialysate and they were only detected because of a very sensitive blood leak monitoring system (3). With the initiation of the industrial production the variation of leaks was found to vary considerably depending on the methodology of irradiation sterilization (3.2 megarad as minimum dose). The first industrial batch of 50 dialysers resulted in 3 evident

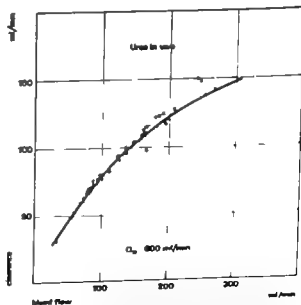


Fig 4 In vivo clearance for urea. The black spots are values with single pass dialysate flow and the open spots indicate local recirculation (7.5 U/ml) added to the mentioned 600 ml fresh dialysate.

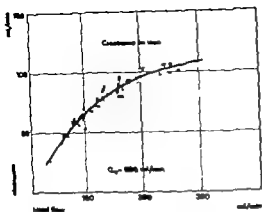


Fig 3 In vivo clearance for creatinine. For further explanation see text.

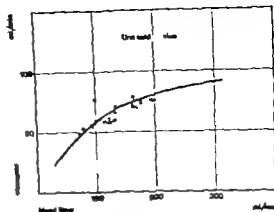


Fig 6 In vivo clearance for uric acid. For further explanation see text.

ruptures necessitating replacement of the dialyser during dialysis in two cases. A further 13 weak transient leaks were detected. By sterilising over longer period of time and carefully adjusting a number of parameters primarily the moisture contents of the membrane the high incidence of the leaks has been eliminated and no blood leak has occurred during the last 500 dialyses.

DISCUSSION

The residual blood volume averaging 3 l ml is acceptable. This is obtained with combined washback with 200-300 ml saline and air. An isolated air inflation without a previous saline washback will result in at least 20-40 ml extra of residual blood being lodged as a very thin (0.004 mm) layer on the entire membrane. As pointed out by others a similar amount of blood is lost in the blood lines (5, 6).

Considerable variations of ultrafiltration rate as shown in Fig. 3 is well known clinical observation. The variations from the average values are more than 10%. The reasons for this fact are not only variations in the membrane properties but also individual differences in patient response in part due to deposits (proteinic or cellular) on the membrane resulting in variable oncotic pressure in the blood-membrane interface.

The bonded membrane area of 0.96 m² results in an efficiency which in practice equals values obtained with parallel plate dialysers with 15-20% larger area. This indicates high specific efficiency.

As it is seen from the clearance values (cf Figs. 4-7) no significant improvement could be discerned

by larger dialysate velocity i.e. recirculation which suggests that the dialysate mixing is well established already at a single pass flow of 600 ml/min.

This irradiation sterilized prototype of a dialyser has been found to be acceptable for clinical use with the sole exception of an unpredictable variation of rupture frequency a problem which has been found to be closely related to the methodology of sterilisation by irradiation. Subsequent experiments have now eliminated this problem for the present generation of the dialyser.

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Supplementum 609

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NEPHROPATHY IN AC COUNTY SWEDEN IN RELATION TO
POPULATION DENSITY AND PREVALENCE OF SMALL RODENTS

By

Kurt Nyström

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POPULATION DENSITY AND PREVALENCE OF SMALL RODENTS

By

Kurt Nyström

From the Department of Medicine (Head: Nils Törnblom)
University of Umeå S-901 87 Umeå 6 Sweden

Acta Medica Scandinavica supplement no

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Kurt Nyström

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4 Information on the prevalence of small rodents in northern Sweden was sought mainly in zoological literature for the period 1959 - 1975. The ratings of different observers for periods (seasons) ranging from May to April the following year were accumulated and the prevalence of animals within each season studied was classified as being either of a low, middle or high/very high grade. When examining the seasons from 1959 and onwards, the prevalence of animals was found to rise and fall in a cyclical manner from a season with a low through a season with a middle to one or two season(s) with a high or very high prevalence of small rodents and always in that order. For the period 1961 - 1974 four such evolutionary cycles of small rodents were discernible.

5 When studied for periods (seasons) each extending from May one year to the following April, i.e. for periods identical to those for which information on prevalence of small rodents was presented in this study, cases of EBN were registered for each of these. The seasonal incidence of the disease was found to range from a minimum of three to a maximum of 95 cases.

The study covers 16 seasons and when they were examined in an unbroken succession, the seasonal incidence of the disease was found to vary in a cyclical manner, each cycle comprising three to four seasons. Within each cycle an increase in number of cases from the first to the last season was found ranging from 400 to 1 600 per cent.

6 When compared season for season, a congruence was found between variations in seasonal incidence documented for cases of EBN in this study and variations in seasonal prevalence of small rodents as rated by different observers and the cyclical variations of the two phenomena studied were found to coincide.

Significant differences were found between the distributions of cases of EBN registered in AC county for seasons with a separate grade of prevalence of small rodents in northern Sweden (low, middle and high or very high).

ABSTRACT

1 Incidence and prevalence of endemic benign (epidemic) nephropathy (EBN) was studied in AC county Sweden for the period 1959 - 1975. The first cases of the disease were described in 1934. Most of the Scandinavian hospitals from which subsequent cases of the disease were reported are situated north of the 60th parallel; AC county forms part of that territory (Fig 1 page 7). The county is situated just south of the Arctic circle and covers a land area of 55 400 sq km. Its mean population for the period studied was 235 000.

2 The mean prevalence for the period studied was 0.08 cases per 1 000 inhabitants and year ($n = 267$). Cases of EBN were diagnosed nearly four times as often among males as among females. The maximum mean yearly prevalence for males 0.28 cases per 1 000 was found among those aged 30-39 (29 per cent of the male cases). For females the corresponding figure 0.07 cases per 1 000 was found among those aged 40-49 (25 per cent of the female cases). Only 2.6 per cent of the cases were children 15 years or younger. The distribution of the population and of cases of EBN on different occupational classes was roughly an equal one. The mean of the registered maximum serum creatinine concentration was higher for females than for males.

3 During the period studied cases appeared in each of the 12 municipal districts of the county. The domiciles of the patients were found to be distributed seemingly at random within individual municipal districts and over the county as a whole.

The mean prevalence of EBN in the sparsely populated area of the county was 1.5 times that of built-up areas and twice that of towns. In the thinly populated inland region it was twice that of the more densely populated coastal region. The prevalence of the disease was much higher in the northernmost than in the southernmost municipal districts.

In the inland region the prevalence of EBN was higher in the eastern than in the western districts.

4 Information on the prevalence of small rodents in northern Sweden was sought mainly in zoological literature for the period 1909 - 1975. The ratings of different observers for periods (seasons) ranging from May to April the following year were accumulated and the prevalence of animals within each season studied was classified as being either of a low middle or high/very high grade. When examining the seasons from 1959 and onwards the prevalence of animals was found to rise and fall in a cyclical manner from a season with a low through a season with a middle to one or two season(s) with a high or very high prevalence of small rodents and always in that order. For the period 1961 - 1974 four such evolutionary cycles of small rodents were discernible.

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6 When compared season for season a congruence was found between variations in seasonal incidence documented for cases of EBN in this study and variations in seasonal prevalence of small rodents as rated by different observers and the cyclical variation of the two phenomena studied were found to coincide.

Significant differences were found between the distributions of cases of EBN registered in AC county for season with a separate grade of prevalence of small rodents in northern Sweden (low middle and high or very high).

7 77 per cent of the cases of EBN diagnosed in AC county during seasons when the prevalence of small rodents in northern Sweden was of a low grade appeared during the first half of these seasons. For seasons when the prevalence of animals was of a middle grade the corresponding figure was 17 per cent of the cases diagnosed during these seasons and for seasons when the prevalence of small rodents was of a high or very high grade cases of EBN were diagnosed in an almost equal proportion during the first and second halves of these seasons.

When cumulating the number of cases of EBN diagnosed during seasons when the prevalence of small rodents was of a separate grade the intercept with the 50th percentile of the distributions obtained for seasons when the prevalence of animals was of a low grade fell on an average four months in advance of that for distributions obtained when the prevalence of animals was of a middle grade; the corresponding intercept for distributions obtained when the prevalence of small rodents was of a high or very high grade fell in between these extremes. These results might possibly be associated with changes in age distribution of animals during the evolutionary cycle of the small rodent population.

8 The main results of this study were interpreted as follows: The congruent variations found between seasonal incidence of endemic benign (epidemic) nephropathy in AC county and seasonal prevalence of small rodents in northern Sweden speak in favour of a close state of dependence between the two phenomena for the period studied. The constantly higher prevalence of the disease among people in AC county living farther apart than among those living more closely grouped demonstrated for the period examined in this study is compatible with such an interpretation.

The random geographic distribution in AC county and its municipal districts of the patients' domiciles, the appearance of cases during every period studied, the low mean prevalence of the disease and the lack of evidence for its spreading from person to person are all findings compatible with the disease studied in this investigation being of an endemic nature in the area(s) examined.

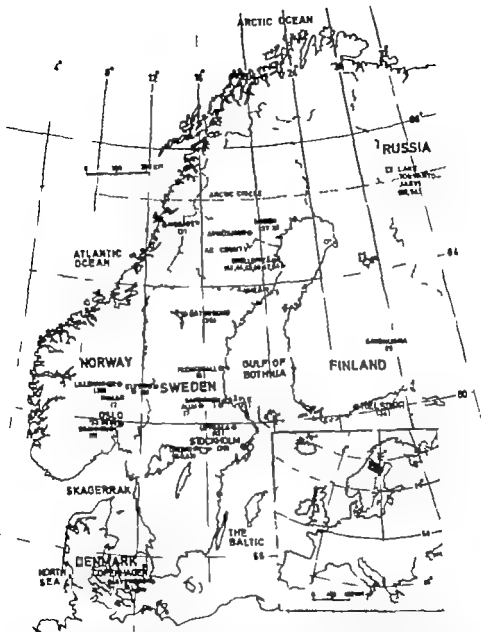


Fig 1 Contour map of Scandinavia showing the geographic position of hospitals from which cases of endemic benign (epidemic) nephropathy have been reported in the literature; references within brackets. Boundaries of AC country where the present study was carried out are indicated (---). For details on distribution of cases in Finland see (29).

■ indicate approximate position of area from which outbreaks were reported among German and Finnish troops in 1942.

Inset map Europe. Hatched area. Position of AC country, Sweden.

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I 2 Etiology of the disease and factors discussed as being of pathogenic importance

The symptoms and signs of EBM are strongly suggestive of the disease being of an infectious origin (37 38 44) However efforts reported by previous investigators to demonstrate an infectious agent in these patients have completely failed (24 29 33 38 39 50 56) but a cytopathic factor is now being investigated (19 43) Although cases of the disease were reported among members of the same family (29 45) among people working and lodging together e.g. lumberers (61) and soldiers (20 54) and among those occupied in medical service (24 61) no evidence was found in favour of the disease being spread by means of personal contacts (54) Most likely EBM is therefore not propagated from person to person (12 29 47) and consequently much attention has been paid to circumstances in the patients immediate surroundings e.g. living or occupational conditions when trying to explain why and how people contract the disease In his report of the outbreak in 1942 among German troops campaigning on the northern stretch of the Finnish Russian border STURMBAUT concluded that close contact with the wet ground was of decisive importance for contracting the disease (54) In papers on epidemic nephropathy several Scandinavian authors (12 32 38 61) point to the circumstance that patients were a considerable who had been living under conditions of hygiene rated as primitive when they were struck by the disease The war also patients who reported having pursued some form of outdoor activity shortly before contracting the disease e.g. fishing or hunting (33) or touring the mountains (32) Such activities as logging (23 24 39 60) hay making (29 40) road (41) or mill road work (21) or picking berries in the woods or on peat land (45) have also been singled out when trying to explain why people contract EBM Such detail concerning the patient activities prior to being struck by the disease is usually given as part of case histories and are thus valid only for individual patients or a fraction of the material presented and it is therefore difficult to evaluate to what extent they might be rated as being of pathogenic importance; this difficulty is enhanced by our ignorance of the incubation time

I INTRODUCTION

In 1934 ZETTERHOLM described seven patients from Skellefteå as cases of acute nephritis simulating acute abdominal disease (64). Shortly afterwards MYHRMAN published an account of likewise seven cases from Östersund of a kidney disease with peculiar symptoms and signs (36). All these patients spontaneously made an uneventful and apparently complete recovery despite having been found to have proteinuria and a raised concentration of non protein serum nitrogen.

Since then a substantial number of authors have reported similar cases. Gradually it became obvious that the great majority of the patients were living in that part of Scandinavia lying north of a line corresponding to the 60th parallel (Fig 1 page 7) and in that area they were found when sought for to be by no means rare (29 44 45 61). Hitherto no cases of a similar kind have been observed in Iceland (59).

I 1 The disease as an entity and its designation

Repeated efforts have completely failed to demonstrate the symptoms and signs of these patients to be the consequence of or associated with any type of disease usually seen in acutely ill individuals displaying signs of a kidney affection (24 29 50 56). Consequently the tentative suggestion (36 38) that this condition constitutes a clinical entity of its own is supported by most authors (15 23 29 45 48).

Following the reports (20 54) of an extensive outbreak among front soldiers on the northern stretch of the Finnish-Russian border of an illness similar to that seen in Sweden MYHRMAN (37) suggested the disease be named epidemic nephropathy. The appropriateness of this designation has however been questioned (6 28 32 46 48) and other denominations have also been proposed (6 15 44). Some authors suggest that the disease might be endemic in northern Scandinavia (40 41 46). The proposal to classify it as a (Scandinavian) variety of (Asian) epidemic hemorrhagic fever (25) with renal syndrome is also advocated (29 45 56 60). The disease is forthwith referred to as endemic benign nephropathy (EBN) for reasons to be presented.

I 2 Etiology of the disease and factors discussed as being of pathogenic importance

The symptoms and signs of EBN are strongly suggestive of the disease being of an infectious origin (37 38 44). However efforts reported by previous investigators to demonstrate an infectious agent in these patients have completely failed (24 29 33 38 39 50 56) but a cytopathic factor is now being investigated (19 43). Although cases of the disease were reported among members of the same family (29 45) among people working and lodging together e.g. lumberers (61) and soldiers (20 54) and among those occupied in medical service (24 61) no evidence was found in favour of the disease being spread by means of person to person contacts (54). Most likely EBN is therefore not propagated from person to person (12 29 47) and consequently much attention has been paid to circumstances in the patients immediate surrounding e.g. living or occupational conditions when trying to explain why and how people contract the disease. In his report of the outbreak in 1942 among German troops campaigning on the northern stretch of the Finnish-Russian border STUHLHAUT concluded that close contact with the wet ground was of decisive importance for contracting the disease (54). In papers on epidemic nephropathy several Scandinavian authors (12 32 38 61) point to the circumstance that patients were encountered who had been living under conditions of hygiene rated as primitive when they were struck by the disease. There were also patients who reported having pursued some form of outdoor activity shortly before contracting the disease e.g. fishing or hunting (33) or touring the mountains (32). Such activities as logging (23 24 39 60) hay-making (29 40) road (41) or rail road work (22) or picking berries in the woods or on peat land (45) have also been singled out when trying to explain why people contract EBN. Such details concerning the patients activities prior to being struck by the disease are usually given as part of case histories and are thus valid only for individual patients or a fraction of the material presented and it is therefore difficult to evaluate to what extent they might be rated as being of pathogenic importance; this difficulty is enhanced by our ignorance of the incubation time.

The outbreak among German troops in 1942 was reported to coincide with the appearance of small rodents in great abundance within the area where the soldiers contracted their disease (54) and in subsequent reports (20 29 45 55 61) examples were presented of cases accumulating during years when voles were seen in great numbers. Being a resident of or visiting those parts of (northern) Scandinavia where small rodents are common was reported (12 38 40 41 45) to increase the risk of contracting the disease and in several Scandinavian studies (9 32 46 50) attention was called to the circumstance that individual patients sometimes reported having been in more or less intimate contact with small rodents before being taken ill.

I 3 Distribution of cases among the population

In a nationwide survey of hospitalized cases of epidemic nephropathy among civilians in Finland (29) 97 per cent of the patients were found to be living in the countryside, a proportion significantly deviating from that (44.3 per cent) of individuals living outside built-up areas for the entire population; 70 per cent of the cases diagnosed in a circumscribed area in the south-easterly part of Finland were farmers or members of farm households. In this district also the majority of the patients, 95 per cent, were living in the countryside.

The sex ratio of EBN among civilians is reported to be highly in favour of men. In a Swedish survey (47) the proportion of men was found to be 89.9 per cent (109 cases) and in Finland (29) the corresponding figure was 80.2 per cent (230 cases).

In a study of cases of EBN diagnosed within the eastern part of AC county, Sweden (47) the cases were found to appear roughly in proportion to the geographic distribution of the population; the fraction of people or of patients living outside built-up areas was not reported, however.

I 4 Number of cases and incidence of the disease as reported in previous studies

For the period 1934 - 1974 Scandinavian authors have reported nearly 1 000 cases of the disease among civilians

Three different kinds of events may be discerned when surveying previous studies on the incidence of the disease

(a) The appearance of sporadic cases apparently isolated from each other (7 11 21 24 33 37 40 41 50 63)

(b) Outbreaks consisting of a varying but moderate number of cases (6 9 12 15 23 31 32 36 38 61 62 64) some occurring within a restricted geographic area To these may be added the singular outbreak among front soldiers on the Finnish-Russian border in 1942 probably numbering more than 2 000 cases by far the most extensive and most concentrated outbreak of the disease recorded (20 54)

(c) A variation in number of cases obvious when the incidence was studied for a sufficiently long period e g a decade or more (29 43 46 47)

An uneven intra-annual distribution of cases was also reported (12 29 34 45) the majority appearing during autumn and early winter Furthermore cases were reported to be more commonly encountered during those periods of the winter seasons when the mean out-door temperature was low i e -15° Centigrad or lower (29)

I 5 Purpose of the present investigation

The purpose of this investigation was

(a) to study incidence and prevalence of endemic benign (epidemic) nephropathy and geographic distribution of cases of the disease in AC county Sweden and its municipal districts

(b) to examine factors that might affect these parameters with emphasis on the influence of population density and prevalence of small rodents

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Table I Diagnoses used for selecting medical records likely to document cases of endemic benign nephropathy (EBN)
Extract from WHO International classification of Diseases Injuries and causes of Death; Scandinavian editions with subdivisions added

1957		1964		1968	
049 2	Infectio acuta UNS	138 19	Infectio UNS	079 98	Infectio virosa NUD
		475 99		136 09	Infectio NUD
481	Influenza UNS	481 90	Influenza UNS	470 99	Influenza NUD
590	Nephritis acuta	590	Nephritis acuta	580 99	Glomerulonephritis acuta
593	Nephritis causa ac vel chron non indicato	593 99	Nephritis causa ac vel chron non indicato	583 99	Nephritis NUD
				590 10	Pyelonephritis acuta
				590 98	Infectio renis alii definita
600	Morbi infectiosi renum	600	Morbi infectiosi renum alia	590 99	Infectio renis NUD
				593 10	Nephrosis acuta tubularis
603	Alii morbi renis et ureteris	603 09	Morbi renis et ureteris alii UNS	593 22	Proteinuria benigna
				593 28	Morbi renis alii
				593 281	Nephropathia endemica benigna (EBN)
				593 287	Nephritis interstitialis
				593 29	Morbi renis NUD
				599 02	Infectiones tractuum urinariorum NUD
				599 09	Alii morbi tractuum urinariorum NUD
786 4	Polyuria	786 40	Polyuria	785 51	Symptomata abdominalia acuta NUD
786 5	Oliguria anuria	786 50	Oliguria	786 40	Polyuria
		786 51	Anuria	786 50	Oliguria neonati excepta
789 0	Albuminuria UNS	786 99	Symptomata organorum urogenitalium alia sive UNS	786 51	Anuria neonati excepta
789 4	Haematuria UNS			788 80	Febris incertae causae
792	Uraemia	789 09	Albuminuria	789 00	Proteinuria (albuminuria)
		789 49	Haematuria	789 30	Haematuria
		789 99	Uraemia	792 99	Uraemia
		793 90	Alii casus morbi organorum urogenitalium suspecti		

II METHODS

II 1 Documentation of cases

From the files of the departments of medicine and pediatrics and that of infectious diseases at the three hospitals in AC county those records were studied which had been made for patients finally diagnosed either as cases of epidemic nephropathy or as some of the conditions most likely to be confused with cases of ERM (Table I page 12)

Irrespective of the actual diagnosis in the records examined it was estimated whether the information obtainable from each one was adequate for accepting the patient as a case of ERM or not; this decision was made using the discriminating characteristics presented in Table II

Table II Criteria used for discriminating cases of ERM

-
- 1 Suddenly falling ill
 - 2 Elevated body temperature
 - 3 Ache and/or pain mostly abdominal or in the back
 - 4 Gastrointestinal symptoms
 - 5 Proteinuria
 - 6 Elevated concentration of serum non protein nitrogen
 - 7 Uneventful course
 - 8 Spontaneous recovery
-

For each case was recorded date of onset of illness and for cases registered from December 1959 to April 1974 also profession, age, sex and geographic position of the patient's domicile; these demographic data and also some of the findings recorded during the acute phase of the disease are presented in the Appendix (Table A)

From May 1960 to April 1974 the geographic site of the domicile was plotted for each patient on maps of AC county (pages 35-41) indicating whether it was situated in a sparsely populated or built up area or in a town. This was done according to the official definition of a built-up area being a place

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593	Nephritis causa ac vel chron non indicato	593 99	Nephritis causa ac vel chron non indicato	583 99 590 10 590 98		Nephritis NUD Pyelonephritis acuta Infectio renis alii definita		
600	Morbi infectiosi renum	600	Morbi infectiosi renum alia	590 99 593 10		Infectio renis NUD Nephrosis acuta tubularis		
603	Alii morbis renis et ureteris	603 09	Morbi renis et ureteris alii UNS	593 22 593 28 593 281 593 287 593 29 599 02 599 09		Proteinuria benigna Morbi renis alii Nephropathia endemica benigna (EBN) Nephritis interstitialis Morbi renis NUD Infectiones tractuum urinariorum NUD Alii morbi tractuum urinariorum NUD		
786 4	Polyuria	786 40	Polyuria	783 31		Symptomata abdominalia acuta NUD		
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II 4 Small rodent field population dynamics

The account presented in this study of small rodent population dynamics was based on information sought in zoological literature (1-5 10 14 16 18 22 26 27 35 49 57) and among ecological zoologists (17 22 58)

II 4 1 General theory

Population dynamics of wild living animal are most extensively studied in small rodents mostly voles. In circumpolar regions of the northern hemisphere field populations of these animals are subject to recurring changes (18 35) which may be summarized in the following way

(a) The number of animals fluctuates in a regular manner

(b) These fluctuations may occur synchronously although with phasic shifts over a vast geographic area (1 16 35 57)

(c) When studied simultaneously an augmented amplitude of the fluctuations is usually observed on more northerly latitudes compared to those found in areas situated more to the south (16 22 27)

(d) It has become an established convention (4 26 35) among ecological zoologists when studying the population dynamics of small rodents to survey periods starting in spring (usually in May) when vegetation begins to sprout and continuing to April the following year. In the present study this accepted custom was observed and such a period is forthwith referred to as a season.

The fluctuations in number of animals to which rodent populations are subject form cyclical events (1 18 35) which are repeated every third to fourth year (1 16 35) four-year and also five-year ones appearing among the dominating three-year cycles (35).

During such an evolutionary cycle of small rodents the quantity of animal in a field population changes in the following order (1 5)

Starting with a season when the number of animals is at its lowest level during the cycle i.e. a season of low abundance there follows a season when the reproductive rate of the population is found to be considerably speeded up. As the ani-

with 200 inhabitants or more and where the distance between buildings does not exceed 200 metres (42) Each site was labeled with the sequential number(s) given to the patient(s) inhabiting it

II 2 Population characteristics of AC county

For each of the 12 municipal districts into which AC county is divided detailed information was obtained for the census years of 1960 1965 and 1970 (42) on land area distribution of population according to sex and age-groups and occupational classes as well as proportion of population living in the sparsely populated and in the built-up areas respectively; for the remaining years of the period studied the number of people living outside and inside built-up areas respectively had to be calculated

Details of AC county built-up areas were obtained from an inventory made by the County Administration's Unit of Regional Economics (30) which included a map showing for 1970 their actual position and also displaying the geographic distribution of the county population in multiples of 10

To account for the very uneven geographic distribution of the county population the prevalence of EBN was studied separately for the inland region comprising the seven municipal districts where the mean population for the period studied was lower than the county mean (section III 1 2 page 20) and correspondingly for the coastal region comprising the five districts where the mean population exceeded that of the county mean for the period (section III 1 3 page 22)

II 3 Statistical methods

Most of the findings in this study were analyzed using non-parametric statistics (13 51 52) Whenever applied the type of test is mentioned in the text

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mals usually continue to breed during the second half of the season also (35) the net result will be an increase in number of voles their quantity reaching middle abundance During the following seasons the number of animals increases still further reaching high or very high abundance The number of animals may remain high also for the season to come in which case a four year cycle is formed Usually however maximum (peak) number of small rodents is reached during the third season of the evolutionary cycle

When the number of animals exceeds a critical level a change in their behaviour called dispersal is observed (4 35) consisting in a great proportion of the animals migrating into surroundings usually not inhabited by them The maximum density attained by the small rodent population varies from one evolutionary cycle to another and dispersal is more pronounced when very high levels of animals are reached (1) thus increasing the chance of their existence being generally observed creating the impression of a rodent year

In seasons of high or very high abundance the small rodent population's reproductive capacity is reported to decline as early as during the first half of the season and consequently the population increase is halted (1 22 58) During the latter half of such a season the balance of the population is shifted for reasons hitherto obscure (1 35) and usually most of the population is wiped out because of a tremendous increase in mortality - a population crash (4 35); the stage is thus set for another evolutionary cycle to begin

II 4 2 Field population dynamics of small rodents in northern Sweden

The information on the prevalence of small rodents in northern Sweden was obtained either as ratings of the change in quantity of animals taking place between successive seasons the observers using verbal descriptions and classifying the seasonal prevalence of animals as being either low middle or high/very high - peak (10 16 17 26 27 58) or as quantitative information mostly as number of animals obtained at open field catches (14 16 22); this information was used to

indicate the change in seasonal prevalence of small rodents that took place from one season to the next

As no single observer reported observations covering every season of the period studied in this section 1957 - 1975 the varying number of individual observations for each season were accumulated and expressed verbally using the classification accounted for in the opening paragraph of this section

II Climate

Information on weather conditions each year in AC county for the period 1960 - 1974 was collected for the following meteorological variables (42); the data were omitted

- 1 date of the first and last day with continuously snow-covered ground;
- 2 number of days with snow-covered ground;
- 3 maximum depth of the snow-cover in cm;
- 4 number of day when the ground was free of snow
- 5 maximum and minimum amount of precipitation in mm for every month periods with and those without snow-cover being studied separately;
- 6 mean amount of yearly precipitation in mm;
- 7 maximum and minimum outdoor temperature in degree Centigrade for every month of the period

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III RESULTS

III 1 Prevalence of EBN in relation to differences in population density

III 1 1 AC county

Cases of EBN were recorded for every year of the period studied (Table XI page 44). The yearly prevalence of the disease (Table III page 19) was found to vary considerably but in a recurring fashion attaining maximum figures in 1963, 1966 and 1970. The prevalence among people living in built-up areas varied in synchrony with the prevalence among those inhabiting the sparsely populated area of the county.

The mean prevalence for the period studied was 0.071 cases per 1 000 inhabitants and year (range 0.009-0.329). In built-up areas containing 60.7 per cent of the mean county population for the period examined the corresponding figure was 0.059 (0.0284) and in the sparsely populated area where 39.3 per cent of the mean population was resident it was 0.090 (0.011-0.428) a mean prevalence for the sparsely populated area 1.5 times that of the built-up ones.

In the sparsely populated area of the county the population diminished by 36.9 per cent during the period studied while it increased by 29.9 per cent in the built-up areas. The entire county population decreased by only 2 per cent during the same period. Consequently the proportion of the population living in the sparsely populated area of the county diminished during the period examined from 48 per cent in 1960 to 31 per cent in 1973.

A higher prevalence of EBN was registered among people living in the sparsely populated area than among those inhabiting the built-up areas for 12 of the 14 years studied (Table VI page 24). This difference was significant when tested statistically applying a one-sided hypothesis ($p < 0.005$ using Wilcoxon's signed rank test for paired differences).

T b1 If AC county prevalence of RMS 1960 1973 among people living outside and those living inside built areas Number of cases per 1 000 inhabitant and year

Year	Site of domicile			built up areas			AC county		
	parly populated area								
	Number of inhabitants x 1 000	cases	Cases per 1 000 in- habitants	Number of inhabitants x 1 000	cases	Cases per 1 000 in- habitants	Number of inhabitants x 1 000	cases	Cases per 1 000 in- habitants
1960	114 8	6	0 052	124 6	3	0 024	239 4	9	0 028
61	111 6	6	0 054	128 1	0	0	239 7	6	0 025
62	108 2	8	0 074	130 4	4	0 031	238 6	12	0 050
63	103 6	11	0 106	133 2	5	0 038	236 8	16	0 068
64	100 0	5	0 050	135 0	2	0 015	235 0	7	0 030
1965	98 06	3	0 031	137 3	4	0 029	233 4	7	0 030
66	93 49	15	0 160	140 4	15	0 107	233 9	10	0 128
67	90 63	3	0 033	143 9	6	0 042	234 5	9	0 038
68	88 36	1	0 011	146 9	1	0 007	235 2	2	0 009
69	84 32	9	0 106	150 3	12	0 080	234 8	21	0 089
1970	80 11	14	0 175	153 1	13	0 085	233 2	27	0 116
71	77 09	3	0 039	156 0	5	0 032	233 1	8	0 034
72	74 23	1	0 013	159 4	3	0 019	233 6	4	0 017
73	72 44	31	0 428	161 8	46	0 284	234 2	77	0 329
Total	116	119		119			235		
loan	92 51	8 29	0 090	142 9	8 50	0 059	235 4	16 79	0 071

III RESULTS

III 1 Prevalence of EBN in relation to differences in population density

III 1 1 AC county

Cases of EBN were recorded for every year of the period studied (Table XI page 44) The yearly prevalence of the disease (Table III page 19) was found to vary considerably but in a recurring fashion attaining maximum figures in 1963 1966 and 1970 The prevalence among people living in built-up areas varied in synchrony with the prevalence among those inhabiting the sparsely populated area of the county

The mean prevalence for the period studied was 0 071 cases per 1 000 inhabitants and year (range 0 009-0 329) In built-up areas containing 60 7 per cent of the mean county population for the period examined the corresponding figure was 0 059 (0-0 284) and in the sparsely populated area where 39 3 per cent of the mean population was resident it was 0 090 (0 011-0 428) a mean prevalence for the sparsely populated area 1 5 times that of the built-up ones

In the sparsely populated area of the county the population diminished by 36 9 per cent during the period studied while it increased by 29 9 per cent in the built-up areas The entire county population decreased by only 2 per cent during the same period Consequently the proportion of the population living in the sparsely populated area of the county diminished during the period examined from 48 per cent in 1960 to 31 per cent in 1973

A higher prevalence of EBN was registered among people living in the sparsely populated area than among those inhabiting the built-up areas for 12 of the 14 years studied (Table VI page 24) This difference was significant when tested statistically applying a one-sided hypothesis ($p < 0 005$ using Wilcoxon's signed rank test for paired differences)

Table III AC county prevalence of EDW 1960 1973 among people living outside and those living inside built-up areas Number of cases per 1 000 inhabitants and year

Year	Site of domicile				built-up areas				AC county			
	sparsely populated area											
	Number of inhabitants x 1 000	cases	Cases per 1 000 inhabitants	Number of inhabitants x 1 000	case	Cases per 1 000 inhabitants	Number of inhabitants x 1 000	cases	Number of inhabitants x 1 000	cases	Cases per 1 000 inhabitants	
1960	114 8	6	0 052	124 6	3	0 024	239 4	9	239 4	9	0 028	
61	111 6	6	0 054	128 1	0	0	239 7	6	239 7	6	0 025	
62	108 2	8	0 074	130 4	4	0 031	238 6	12	238 6	12	0 050	
63	103 6	11	0 106	133 2	5	0 038	236 8	16	236 8	16	0 068	
64	100 0	5	0 050	135 0	2	0 015	235 0	7	235 0	7	0 030	
1965	96 06	3	0 031	137 3	4	0 029	233 4	7	233 4	7	0 030	
66	93 49	15	0 160	140 4	15	0 107	33 9	30	33 9	30	0 128	
67	90 63	3	0 033	143 9	6	0 042	234 5	9	234 5	9	0 038	
68	88 36	1	0 011	146 9	1	0 007	235 2	2	235 2	2	0 009	
69	84 51	9	0 106	150 3	12	0 080	234 8	21	234 8	21	0 089	
1970	80 11	14	0 175	153 1	13	0 085	233 2	27	233 2	27	0 116	
71	77 09	3	0 039	156 0	5	0 032	233 1	8	233 1	8	0 034	
72	74 23	1	0 013	159 4	3	0 019	233 6	4	233 6	4	0 017	
73	72 44	31	0 428	161 8	46	0 284	234 2	77	234 2	77	0 329	
Total	116			119			235		235			
Mean	92 51	8 29	0 090	142 9	8 58	0 059	235 4	16 9	235 4	16 9	0 071	

III RESULTS

III 1 Prevalence of EBN in relation to differences in population density

III 1 1 AC county

Cases of EBN were recorded for every year of the period studied (Table XI page 44). The yearly prevalence of the disease (Table III page 19) was found to vary considerably but in a recurring fashion attaining maximum figures in 1963, 1966 and 1970. The prevalence among people living in built-up areas varied in synchrony with the prevalence among those inhabiting the sparsely populated area of the county.

The mean prevalence for the period studied was 0.071 cases per 1 000 inhabitants and year (range 0.009-0.329). In built-up areas containing 60.7 per cent of the mean county population for the period examined the corresponding figure was 0.059 (0.0284) and in the sparsely populated area where 39.3 per cent of the mean population was resident it was 0.090 (0.011-0.428). A mean prevalence for the sparsely populated area 1.5 times that of the built-up ones.

In the sparsely populated area of the county the population diminished by 36.9 per cent during the period studied while it increased by 29.9 per cent in the built-up areas. The entire county population decreased by only 2 per cent during the same period. Consequently the proportion of the population living in the sparsely populated area of the county diminished during the period examined from 48 per cent in 1960 to 31 per cent in 1973.

A higher prevalence of EBN was registered among people living in the sparsely populated area than among those inhabiting the built-up areas for 12 of the 14 years studied (Table VI page 24). This difference was significant when tested statistically applying a one-sided hypothesis ($p < 0.005$ using Wilcoxon's signed rank test for paired differences).

Table III AC county Prevalence of EM 1960 1973 among people living outside and those living inside built P rea Number of case per 1 000 inhabitants and year

Year	Sit of domile			built P rea			AC county		
	apsaely populated are			Number of			Number of		
	Number of	case	Cases per	Number of	case	Cases per	inhabit	cases	Cases per
	inhabit	in 1 000	inhabitants	inhabit	in 1 000	inhabitants	x 1 000	x 1 000	1 000 in-
	x 1 000		h	x 1 000		h			habitants
1960	114 8	6	0 052	124 6	3	0 024	239 4	9	0 028
61	111 6	8	0 054	128 1	0	0	239 7	6	0 025
62	108 2	8	0 074	130 4	4	0 031	238 6	12	0 050
63	103 6	11	0 106	133 2	5	0 038	236 8	16	0 068
64	100 0	5	0 050	135 0	2	0 015	235 0	7	0 030
1965	96 06	3	0 031	137 3	4	0 029	233 4	7	0 030
66	93 49	15	0 160	140 4	15	0 107	233 9	30	0 128
67	90 63	3	0 033	143 9	6	0 042	234 5	9	0 038
68	88 36	1	0 011	146 9	1	0 007	235 2	2	0 009
69	84 51	9	0 106	150 3	12	0 080	234 8	21	0 089
1970	80 11	14	0 175	153 1	13	0 085	233 8	27	0 116
71	77 08	3	0 039	156 0	5	0 032	233 1	8	0 034
72	74 23	1	0 013	159 4	3	0 019	233 6	4	0 017
73	72 44	31	0 428	161 8	46	0 284	234 2	77	0 329
Total	116	116		119	119		235	235	
Mean	92 51	8 23	0 090	142 9	8 50	0 059	235 4	16 73	0 071

III RESULTS

III 1 Prevalence of EBN in relation to differences in population density

III 1 1 AC county

Cases of EBN were recorded for every year of the period studied (Table XI page 44) The yearly prevalence of the disease (Table III page 19) was found to vary considerably but in a recurring fashion attaining maximum figures in 1963 1966 and 1970 The prevalence among people living in built-up areas varied in synchrony with the prevalence among those inhabiting the sparsely populated area of the county

The mean prevalence for the period studied was 0 071 cases per 1 000 inhabitants and year (range 0 009-0 329) In built-up areas containing 60 7 per cent of the mean county population for the period examined the corresponding figure was 0 059 (0-0 284) and in the sparsely populated area where 39 3 per cent of the mean population was resident it was 0 090 (0 011-0 428) a mean prevalence for the sparsely populated area 1 5 times that of the built-up ones

In the sparsely populated area of the county the population diminished by 36 9 per cent during the period studied while it increased by 29 9 per cent in the built-up areas The entire county population decreased by only 2 per cent during the same period Consequently the proportion of the population living in the sparsely populated area of the county diminished during the period examined from 48 per cent in 1960 to 31 per cent in 1973

A higher prevalence of EBN was registered among people living in the sparsely populated area than among those inhabiting the built-up areas for 12 of the 14 years studied (Table VI page 24) This difference was significant when tested statistically applying a one-sided hypothesis ($p < 0 005$ using Wilcoxon's signed rank test for paired differences)

Table IV Inland region Prevalence of Bm 1960 1973 in the seven municipal districts of AC county in which the population density for the period fell below that of the county mean Number of cases per 1 000 inhabitants and year among people living outside and those living inside the built up areas

Year	State of domicile				Built up areas				Inland region			
	Rural population		Total population		Number of		Cases per		Number of		Cases per	
	Inhabitants x 1 000	Cases	Inhabitants x 1 000	Cases	Inhabitants x 1 000	Cases	Inhabitants x 1 000	Cases	Inhabitants x 1 000	Cases	Inhabitants x 1 000	Cases
1960	49 71	2	0 040		29 34	0	0		79 05	2	0 025	
61	48 20	4	0 083		29 90	0	0		78 10	4	0 051	
62	46 50	5	0 108		29 80	1	0 034		76 30	6	0 079	
63	45 58	8	0 176		30 91	3	0 097		76 50	11	0 144	
64	43 71	2	0 023		30 90	1	0 032		74 61	2	0 027	
1965	40 83	1	0 024		29 94	1	0 033		70 77	2	0 028	
66	40 28	9	0 223		30 60	8	0 261		70 88	17	0 240	
67	38 98	1	0 026		30 77	2	0 065		69 73	3	0 043	
68	37 86	2	0 026		30 96	0	0		68 82	1	0 015	
69	36 11	7	0 194		31 45	3	0 095		67 56	10	0 148	
1970	33 05	7	0 212		31 48	6	0 191		64 53	13	0 201	
71	31 40	1	0 032		32 00	1	0 031		63 40	3	0 032	
72	30 43	1	0 033		32 56	1	0 031		63 01	2	0 032	
73	29 44	20	0 679		33 10	16	0 483		62 54	36	0 576	
Total	68				43				111			
Mean	39 43	4 86	0 123		30 98	5 07	0 099		70 41	7 93	0 113	

III 1 2 The inland region

The inland region was inhabited by 29.4 per cent of the mean county population for the period studied (being dispersed over an area of 41 964 sq km (75.8 per cent of the county land area). The mean population density of the seven municipal districts of the region was 1.7 inhabitants per sq km (range 0.1 - 3.4) being 4.2 for the entire county (Appendix Table C).

The prevalence of EBN within the region was found to vary as for the whole county (section III 1 1) attaining maximum values in 1963, 1966 and 1970 the variations in the sparsely populated area and in the built-up ones being congruent (Table IV page 21).

The mean prevalence of EBN in the region for the period studied was 0.113 cases per 1 000 inhabitants and year (range 0.015-0.576) a figure 1.6 times higher than the corresponding one for the whole county. In built-up areas of the region the mean prevalence was 0.099 (0-0.483) and in the sparsely populated area it was 0.123 (0.023-0.679) the latter figure being 1.2 times higher than the former.

The population of the entire region diminished during the period studied by 20.9 per cent and that of its sparsely populated area by 40.8 per cent while it increased in the built-up areas of the region by 12.8 per cent. The proportion of the population living in the sparsely populated area of the region diminished from 62.9 per cent in 1960 to 47.1 per cent in 1973 i.e. by 15.8 per cent while the proportion inhabiting the built-up areas increased as much from 37.1 to 52.9 per cent.

A higher prevalence of EBN was registered among people living outside than among those living inside the built-up areas of the inland region for 10 of the 14 years studied (Table VI page 24). This difference was significant when tested statistically applying a one-sided hypothesis ($p < 0.05$ using Wilcoxon's signed rank test for paired differences).

Tabl IV Inland region on prevalence of RM 1960 1973 in the seven municipalities of AC county in which the mean population density for the period fell below that of the county mean. Number of cases per 1 000 inhabitants and y among people living outside and those living inside the built up areas

Year	Sit f domicilio				built up				inland region			
	per city population				Number of				Number of			
	inhabit	case	case per	Case per	inhabit	cases	Cases per	inhabit	cases	cases	cases per	Case per
	x 1 000		1 000 in	1 000 in	x 1 000		1 000 in	x 1 000		x 1 000	1 000 in	1 000 in
			habitant	habitant			habitant				habitant	habitant
1960	49 72	2	0 040		29 34	0	0	79 05	2	0 025		
61	48 20	4	0 083		29 90	0	0	78 10	4	0 051		
62	46 50	5	0 108		29 80	1	0 034	76 30	6	0 079		
63	45 58	8	0 176		30 91	3	0 097	76 50	11	0 144		
64	43 71	1	0 023		30 90	1	0 032	74 61	2	0 027		
1965	40 83	1	0 024		29 94	1	0 033	70 77	2	0 028		
66	40 28	9	0 223		30 60	8	0 261	70 88	17	0 240		
67	38 96	1	0 026		30 77	2	0 065	69 73	3	0 043		
68	37 86	1	0 026		30 96	0	0	68 62	1	0 015		
69	36 11	7	0 194		31 45	8	0 095	67 56	10	0 148		
1970	33 05	7	0 212		31 48	6	0 191	64 53	13	0 201		
71	31 40	1	0 032		32 00	1	0 031	63 40	2	0 032		
72	30 43	1	0 033		32 58	1	0 031	63 01	2	0 032		
73	29 44	20	0 679		33 10	16	0 483	62 54	36	0 576		
Total		68				43			111			
Mean	39 43	4 86	0 123		30 98	3 07	0 099	70 41	7 93	0 113		

III 1 3 The coastal region

The coastal region of AC county covers an area of 13 432 sq km (24.2 per cent of the county land area) and was inhabited by 70.6 per cent of the mean county population for the period studied. The mean population density of the five municipal districts of the region was 12.2 inhabitants per sq km (range 5.6-27.9 Appendix Table C). The prevalence of EBN in the sparsely populated area of the region attained maximum values in 1964, 1966 and 1970 and in the built-up ones in 1962, 1966 and 1969 (Table V page 23). For the whole region maximum prevalence figures of EBN were attained in 1962, 1966 and 1970 i.e. for the same years as for the inland region (section III 1 2) and for the entire county (section III 1 1) with 1962 as an exception.

The mean prevalence of EBN for the whole region was 0.054 cases per 1 000 inhabitants and year for the period studied (range 0.006-0.239) a figure 0.76 of the county mean and 1.48 of that for the inland region. In built-up areas the corresponding figure was 0.049 (0-0.233) and in the sparsely populated area it was 0.065 (0-0.256) the latter figure being 1.3 times as high as the former. For the entire region a population increase of 7.1 per cent was noted for the period studied and for the built-up areas of the region the population figure rose by 35.2 per cent. For the sparsely populated area the population decreased by 34 per cent.

The proportion of the population inhabiting the sparsely populated area of the region diminished by 15.1 per cent during the period studied from 40.1 per cent in 1960 to 25.0 per cent in 1973 and rose for the built-up areas by a corresponding amount from 59.4 per cent in 1960 to 75.0 per cent in 1973.

A higher prevalence of EBN was registered among people living outside than among those living inside the built-up areas of the coastal region for 11 of the 14 years studied (Table VI page 24) a significant difference when tested statistically applying a one-sided hypothesis ($p < 0.025$ using Wilcoxon's signed rank test for paired differences).

Table V Coastal region prevalence of ERM 1960 1973 in the five municipal districts of AC county in which the mean population density for the period fell above that of the county mean Number of cases per 1 000 inhabitants and year among people living outside and those living inside the built up areas

Year	Site of domicile				built up areas				coastal region			
	sparsely populated areas				Number of				Number of			
	Number of		Cases per		inhabit		cases		inhabit		cases	
	x 1 000	cases	1 000 in-	habitant	x 1 000	cases	1 000 in-	habitant	x 1 000	cases	1 000 in-	habitant
1960	65 11	4	0 061		95 19	3	0 032		160 3	7	0 044	
61	63 40	2	0 032		98 20	0	0		161 6	2	0 012	
62	61 70	3	0 049		100 6	3	0 030		162 3	6	0 037	
63	58 01	3	0 052		102 3	2	0 030		160 3	5	0 031	
64	56 32	4	0 071		104 1	1	0 010		160 4	5	0 031	
1965	55 23	2	0 036		107 4	3	0 028		162 6	5	0 031	
66	53 21	6	0 113		109 8		0 064		163 0	13	0 080	
67	51 67	2	0 039		113 1	4	0 035		164 8	6	0 036	
68	50 50	0	0		115 9	1	0 009		166 4	1	0 006	
69	48 40	2	0 041		118 8	9	0 076		167 2	11	0 066	
1970	47 06	7	0 149		121 6	7	0 058		168 7	14	0 083	
71	45 69	2	0 044		124 0	4	0 032		169 7	6	0 035	
72	43 60	2	0		126 8	2	0 016		170 6	2	0 012	
73	43 00	11	0 256		128 7	30	0 233		171 7	41	0 239	
Total		48				76				124		
Mean	53 08	3 43	0 065		111 9	5 43	0 049		165 0	8 86	0 054	

III 1 3 The coastal region

The coastal region of AC county covers an area of 13 432 sq km (24.2 per cent of the county land area) and was inhabited by 70.6 per cent of the mean county population for the period studied. The mean population density of the five municipal districts of the region was 12.2 inhabitants per sq km (range 6.6-27.9 Appendix Table C). The prevalence of EBN in the sparsely populated area of the region attained maximum values in 1964, 1966 and 1970 and in the built-up ones in 1962, 1966 and 1969 (Table V, page 23). For the whole region maximum prevalence figures of EBN were attained in 1962, 1966 and 1970, i.e. for the same years as for the inland region (section III 1 2) and for the entire county (section III 1 1) with 1962 as an exception.

The mean prevalence of EBN for the whole region was 0.054 cases per 1 000 inhabitants and year for the period studied (range 0.006-0.239), a figure 0.76 of the county mean and 0.48 of that for the inland region. In built-up areas the corresponding figure was 0.049 (0-0.233) and in the sparsely populated area it was 0.065 (0-0.256), the latter figure being 1.3 times as high as the former. For the entire region a population increase of 7.1 per cent was noted for the period studied and for the built-up areas of the region the population figure rose by 35.2 per cent. For the sparsely populated area the population decreased by 34 per cent.

The proportion of the population inhabiting the sparsely populated area of the region diminished by 15.6 per cent during the period studied, from 40.1 per cent in 1960 to 25.0 per cent in 1973, and rose for the built-up areas by a corresponding amount, from 59.4 per cent in 1960 to 75.0 per cent in 1973.

A higher prevalence of EBN was registered among people living outside than among those living inside the built-up areas of the coastal region for 11 of the 14 years studied (Table VI, page 24), a significant difference when tested statistically applying a one-sided hypothesis ($p < 0.025$ using Wilcoxon's signed rank test for paired differences).

III 1 4 Differences between areas and regions of AC county

In Table VI (page 24) are presented in number of cases per 1 000 inhabitants and year the differences in prevalence of EBN analysed in section III 1 1 -3 of this study. Also calculated were those differences obtained when subtracting the prevalence of the disease in the sparsely populated area of the coastal region from that of the inland region ($p < 0.005$) the prevalence of EBN in the built up areas of these regions respectively ($p < 0.02$) and finally the difference in the prevalence of the disease obtained between the entire inland and coastal region ($p < 0.005$).

When studied from December 1959 to April 1974 the mean prevalence of EBN among people living in the thinly populated inland region of AC county was found to exceed that among those inhabiting the more densely populated coastal region. Such was the case not only when studying the prevalence in the sparsely populated area of each region but also when separately investigating built-up areas other than towns and (central parts of) towns respectively of each region (Table VII page 26).

51.3 per cent of the total number of cases of EBN examined in detail were diagnosed among people living in the sparsely populated area of AC county, 30.0 per cent among those inhabiting built-up areas other than towns and 18.7 per cent among town dwellers respectively.

III 2 Distribution of cases according to sex, age-groups and occupational class

III 2 1 Sex

The average ratio of men to women was for the material examined in detail 3.5 being 3.0 for cases diagnosed among people living in the sparsely populated area of the county, 3.2 among town-dwellers and 4.7 for people inhabiting built-up areas other than towns (Table VIII page 27). The ratio between males and females was lowest 2.0 in the only town of the inland region (Lyckeåle) and highest 5.0 among people inhabiting built-up areas other than towns in the coastal region of the county.

ble VI Differences in prevalence of EBN in AC county 1960 - 1973 for areas and regions of different population density as defined in the text Number of cases per 1 000 inhabitants and year
p values denote the probability that the series of differences might be the consequence of chance variation (Wilcoxon's signed rank test for paired differences)

<u>Prevalence of EBN Difference between</u>						
<u>sparsely populated area and built-up areas of</u>						
	AC county	inland region	coastal region	sparsely populated area	built-up areas	the regions
860	0 028	0 040	0 029	-0 021	-0 032	-0 019
61	0 054	0 083	0 032	0 051	0	0 039
62	0 043	0 074	0 019	0 059	0 004	0 042
63	0 068	0 079	0 032	0 124	0 077	0 113
64	0 035	-0 009	0 061	-0 048	0 022	-0 004
965	0 002	-0 009	0 008	-0 012	0 005	-0 003
66	0 053	-0 038	0 049	0 110	0 197	0 160
67	-0 009	-0 039	0 004	-0 013	0 030	0 007
68	0 004	0 026	-0 009	0 026	-0 009	0 009
69	0 026	0 099	-0 035	0 153	0 019	0 082
970	0 090	0 021	0 091	0 063	0 133	0 118
71	0 007	0 001	0 012	-0 012	-0 001	-0 003
72	-0 006	0 002	-0 016	0 033	0 015	0 020
73	0 144	0 196	0 023	0 423	0 250	0 337
P	0 005	< 0 05	< 0 025	< 0 005	< 0 025	< 0 005

III 1 4 Differences between areas and regions of AC county

In Table VI (page 24) are presented in number of cases per 1 000 inhabitants and year the differences in prevalence of EBM analysed in section III 1 1 -3 of this study. Also calculated were those differences obtained when subtracting the prevalence of the disease in the sparsely populated area of the coastal region from that of the inland region ($p < 0.005$) the prevalence of EBM in the built up areas of these regions respectively ($p < 0.025$) and finally the difference in the prevalence of the disease obtained between the entire inland and coastal region ($p < 0.005$).

When studied from December 1959 to April 1974 the mean prevalence of EBM among people living in the thinly populated inland region of AC county was found to exceed that among those inhabiting the more densely populated coastal region. Such was the case not only when studying the prevalence in the sparsely populated area of each region but also when separately investigating built up areas other than towns and (central parts of) towns respectively of each region (Table VII page 26).

51.3 per cent of the total number of cases of EBM examined in detail were diagnosed among people living in the sparsely populated area of AC county. 30.8 per cent among those inhabiting built-up areas other than towns and 18.7 per cent among town-dwellers respectively.

III 2 Distribution of cases according to sex, age-groups and occupational class

III 2 1 Sex

The average ratio of men to women was for the material examined in detail 3.5 being 3.0 for cases diagnosed among people living in the sparsely populated area of the county, 3.2 among town-dwellers and 4.7 for people inhabiting built up areas other than towns (Table VIII page 27). The ratio between male and females was lowest 2.0 in the only town of the inland region (Lycksele) and highest 5.0 among people inhabiting built up areas other than towns in the coastal region of the county.

Table VI Differences in prevalence of EBN in AC county 1960 - 1973 for areas and regions of different population density as defined in the text Number of cases per 1 000 inhabitants and year
p values denote the probability that the series of differences might be the consequence of chance variation (Wilcoxon's signed rank test for paired differences)

<u>Prevalence of EBN Difference between</u>									
<u>sparsely populated area and built-up areas of</u>									
	AC county	inland region	coastal region	<u>inland and coastal region</u>					
				sparsely populated area	built-up areas	the regions			
960	0 028	0 040	0 029	-0 021	-0 032	-0 019			
61	0 054	0 083	0 032	0 051	0	0 039			
62	0 043	0 074	0 019	0 059	0 004	0 042			
63	0 068	0 079	0 032	0 124	0 077	0 113			
64	0 035	-0 009	0 061	-0 048	0 022	-0 004			
965	0 002	-0 009	0 008	-0 012	0 005	-0 003			
66	0 053	-0 038	0 049	0 110	0 197	0 160			
67	-0 009	-0 039	0 004	-0 013	0 030	0 007			
68	0 004	0 026	-0 009	0 026	-0 009	0 009			
69	0 026	0 099	-0 035	0 153	0 019	0 082			
970	0 090	0 021	0 091	0 063	0 133	0 118			
71	0 007	0 001	0 012	-0 012	-0 001	-0 003			
72	-0 006	0 002	-0 016	0 033	0 015	0 020			
73	0 144	0 196	0 023	0 423	0 250	0 337			
P	< 0 005	< 0 05	< 0 025	< 0 005	< 0 025	< 0 005			

Table VIII Total number of men and women diagnosed as cases of EBM in AC county December 1959 - April 1974 in areas and regions of different population density The ratio of males (M) to females (F) is given within brackets

Region	Areas of AC county classified according to population density as being				The entire region/county
	<u>sparsely populated</u>	<u>built-up areas other than towns</u>	<u>towns central parts</u>		
	M/F	M/F	M/F		M/F
Inland	56/16(3 5)	31/7 (4 4)	4/2 (2 0)		91/25(3 6)
Coastal	47/18(2 6)	35/7 (5 0)	34/10(3 4)		116/35(3 3)
AC county	103/34(3 0)	66/14(4 7)	38/12(3 2)		207/60(3 5)

III 2 2 Age-groups

In the lower half of Fig 2 (page 28) is shown separately for each sex the distribution according to age-groups of the mean number of people in the census years of 1960 1965 and 1970 inhabiting the sparsely populated area of AC county and the built up areas respectively (42) Within the different age-groups of both sexes the number of people living in built-up areas was found to exceed the number inhabiting the sparsely populated area of the county male older than 50 years being an exception

In the upper half of Fig 2 we plotted the mean prevalence of EBM as registered from December 1959 to April 1974 among each age-group of people presented in the lower half of the figure The mean prevalence of the disease among the proportion of people living in the sparsely populated area of the county was found to exceed that among the corresponding proportion of people inhabiting the built up areas within each age-group examined for females as well as for males

able VII Prevalence of EBM in AC county December 1959 - April 1974 among people living outside those living inside built-up areas other than towns and among town-dwellers respectively in regions of varying mean population density; mean number of cases per 1 000 inhabitants and year for the period studied

Areas of AC county classified according to their population density as being

Region	sparsely populated			built-up areas other than towns			towns			central parts			The entire region/ county		
	Mean number of in-habit x 1 000	Total number of cases	Mean yearly prevalence	Mean number of in-habit x 1 000	Total number of cases	Mean yearly prevalence	Mean number of in-habit x 1 000	Total number of cases	Mean yearly prevalence	Mean number of in-habit x 1 000	Total number of cases	Mean yearly prevalence	Mean number of in-habit x 1 000	Total number of cases	Mean yearly prevalence
Inland	41.2	72	0.121	23.3	38	0.113	7.0	6	0.060	71.5	116	0.113			
Coastal	55.8	65	0.081	44.9	42	0.065	63.2	44	0.048	163.9	151	0.064			
AC county	97.0	137	0.098	68.1	80	0.081	70.2	50	0.049	235.4	267	0.079			

For the sparsely populated area of the county the mean prevalence of the disease was found for both sexes to increase with increasing age of the patients attaining a maximum for females as well as for males aged 30-39 years then diminishing gradually as the age of the patients increased further. For the built up areas the mean prevalence among males also rose gradually reaching a maximum among men aged 20-29 years then diminishing; the distribution obtained for females in built-up areas was bimodal.

The maximum mean prevalence for males = 282 cases per 1 000 men and year was observed among those aged 30-39 years; 29 per cent of the male cases were diagnosed in this age-group which made up 12.3 per cent of the mean male population for the period studied. The corresponding prevalence figure for females 0.068 was found among those aged 40-49 years 25 per cent of the female cases were diagnosed in that age-group which constituted 13.3 per cent of the mean female population for the period (Appendix Table B).

When surveying within the different age-groups examined the ratio of the proportion of cases of EBN to the corresponding proportion of the mean county population for the period studied cases were found to be proportionately more often diagnosed among people 20-49 years of age and proportionately less often among those younger than 20 or older than 49. With some exceptions this was the case for females as well as for males also when studying the ratio separately for members of both sexes living outside and for those living inside built-up areas (Table IX page 30 Appendix Table B).

III 2.3 Occupation

72.7 per cent of the cases of EBN in AC county were diagnosed among that proportion of the population professionally engaged in occupations of some kind when struck by the disease (Table X page 31 item 1.6). For these occupational classes the proportion of cases of EBN exceeded the proportion they amounted to of the entire county population people engaged in medical service being an exception.

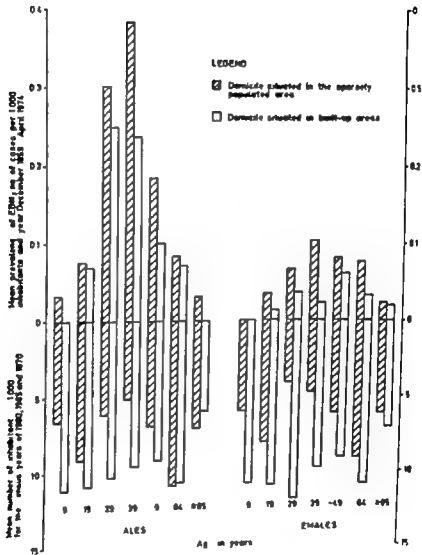


Fig 2 Distribution of the county population for the census years of 1960 1965 and 1970 (lower half) and of the prevalence of EBN in AC county December 1959 - April 1974 in number of cases per 1 000 inhabitants and year (upper half) Mean values for each age-group examined and for males and females living outside and inside built-up areas respectively,

For the sparsely populated area of the county the mean prevalence of the disease was found for both sexes to increase with increasing age of the patients attaining a maximum for females as well as for males aged 30-39 years then diminishing gradually as the age of the patients increased further. For the built up areas the mean prevalence among males also rose gradually reaching a maximum among men aged 20-29 years then diminishing; the distribution obtained for females in built-up areas was bimodal.

The maximum mean prevalence for males 0.282 cases per 1,000 men and year was observed among those aged 30-39 years; 38 per cent of the male cases were diagnosed in this age-group which made up 12.3 per cent of the mean male population for the period studied. The corresponding prevalence figure for females 0.068 was found among those aged 40-49 years 2.1 per cent of the female cases were diagnosed in that age-group which constituted 13.3 per cent of the mean female population for the period (Appendix Table B).

When surveying within the different age-groups examined the ratio of the proportion of cases of EBM to the corresponding proportion of the mean county population for the period studied cases were found to be proportionately more often diagnosed among people 20-49 years of age and proportionately less often among those younger than 20 or older than 49. With some exception this was the case for females as well as for males also when studying the ratio separately for members of both sexes living outside and for those living inside built-up areas (Table IX page 30 Appendix Table B).

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Table IX Ratio of proportion of cases of EBN diagnosed December 1959 - April 1974 to proportion of county population (mean for 1960 1965 and 1970) for males and females in different age-groups living outside (SP) and inside (BU) built-up areas in AC county (AC) - the proportion of cases fell below or + above that of the population
For details see Appendix (Table B)

Age group	Males			Females			Both sexes		
	Area			Area			Area		
	SP	BU	AC	SP	BU	AC	SP	BU	AC
- 9	-	-	-	-	-	-	-	-	-
- 19	-	-	-	-	-	-	-	-	-
- 29	+	+	+	+	+	+	+	+	+
- 39	+	+	+	+	-	+	+	+	+
- 49	+	-	+	+	+	+	+	+	+
- 64	-	-	-	+	+	+	-	-	-
≥ 65	-	-	-	-	-	-	-	-	-

III 3 Distribution of maximum serum creatinine concentration

During the acute phase of EBN a transient rise in concentration of the components forming the serum non-protein nitrogen was registered for 93.7 per cent of the males and for 96.7 per cent of the females (Appendix Table A). Fig. 3 (page 32) shows the distribution between the sexes of the maximum registered creatinine concentration in mg per 100 ml of serum. The mean values for males did not differ between men inhabiting the sparsely populated area of AC county and those living in the built-up ones, nor was any difference in corresponding mean values found between women living in these two areas of different population density.

A higher mean of the maximum serum creatinine concentration registered during the acute phase of the disease was found among females than among males, both for that part of the population inhabiting the sparsely populated area of the county and

Table X Distribution of cases of EBN diagnosed in AC county
December 1959 - April 1974 among different categories
of the county population

Population			Cases of EBN	
Occupation or activity	number	per cent	number	per cent
1 Forestry and farming	17 156	7 4	50	18 7
2 Manufacturing industry and equivalent branches	20 694	8 9	52	19 5
3 Construction	10 394	4 5	20	7 5
4 Commerce and trade commu- nication private services	26 229	11 2	41	15 4
5 Public administration and service	10 394	4 5	24	9 0
6 Med cal service	6 443	2 8	7	2 6

Total 1 6	91 300	39 3	194	72 7
7 Students	47 600	20 4	18	6 7
8 Housewiv s	36 400	15 6	32	12 0
9 Unspecified and no information	4 700	2 0	16	6 0

Total 7 9	88 700	38 0	66	24 7
10 Child en < 1 years	53 200	22 7	7	2 6

Total 1 10	233 200	100 0	267	100 0

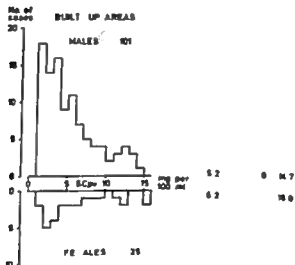
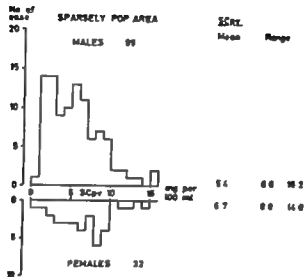


Fig 3 Maximum concentration of creatinine registered in mg per 100 ml of serum (SCpv) Distribution among male and female cases of EBN diagnosed in AC county December 1959 - April 1974 for those inhabiting the sparsely populated area of the county and those living in the built-up ones

for that part living in the built-up ones. This difference between the sexes was significant when tested statistically ($p < 0.001$ using Student's t test).

III 4 Geographic distribution of cases

Figs 4-8 (pages 35-41) show the geographic position of the domicile for each patient diagnosed as a case of EBN in AC county from May 1960 to April 1974 for seasons each of which last from May one year to the following April.

The domiciles were found to be scattered over areas of very different sizes. The 12 cases diagnosed during season 1960-61 (Fig 4 page 35) and the seven diagnosed during season 1968-69 (Fig 7b page 39) appeared among the inhabitants of two of the county's twelve municipal districts situated in the eastern part of the county and bordering on each other. During the majority of seasons the domiciles were spread out fairly evenly over the entire county even during seasons when comparatively few cases were diagnosed (Fig 5a page 35 Fig 7a page 38).

When surveying the chronological order in which the cases appeared during each season studied, no geographic concentration of cases in the sense of localized outbreaks could be discerned.

Four instances where family members contracted EBN within short time of one another were encountered (Appendix Table A). Cases 75 and 80 and 129 and 131 were brothers who fell ill with a two week interval of one another; cases 174, 176 and 177 were son, mother and father who contracted EBN within an interval of one and a half and one week respectively; and cases 22 and 231 were father and daughter who fell ill within one week of one another. All these cases were living in the sparsely populated area of the county.

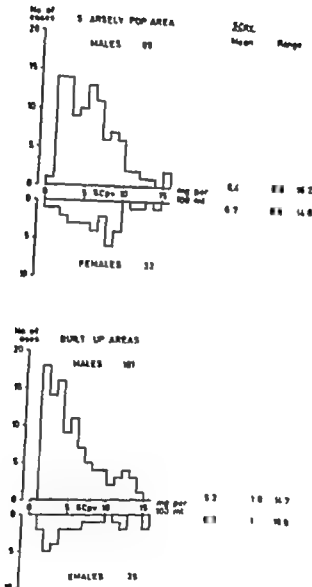


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The mean prevalence of EBN within each of the county's twelve municipal districts was found to vary from 0.02 to 0.21 cases per 1 000 inhabitants and year for the period studied - a ten-fold difference

The mean for the eight districts in the central northern and eastern parts of the county fell above or slightly below the county mean for the period examined whereas for the four districts in the southern part of the county it fell well below that mean. For the seven districts comprising the inland region the mean prevalence of EBN for the period was 1.8 times higher than for the five districts comprising the coastal region (Fig 9 page 42 Appendix Table C)

Figs 4 -8 (pages 35-41) Geographic positions of the domiciles for cases of EBN diagnosed in AC county May 1960 - April 1974. The sequential number given to each case (Appendix Table A) is attached to the domicile of the patient.

On each map are plotted cases of EBN diagnosed during one season lasting from May one year to the following April (cf section II 4 and III 6 -7)

The actual prevalence of small rodents in northern Sweden during each season of the period studied (Table XII page 48) is indicated successively within the quadrangles above the map: L - low M - middle H - high and VH - very high

Domicile situated in the sparsely populated area of the county (x) in a built-up area other than town (o) or in a town (•)

SEASON 1980 1981 12 Cases nos. 3 14

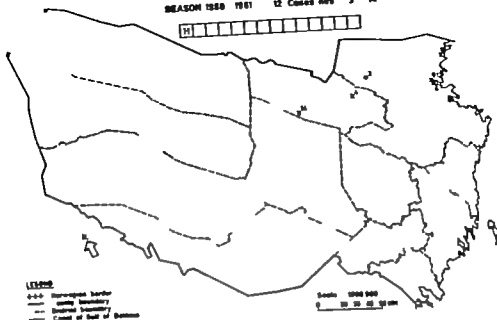
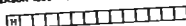


Fig 4; legend on page 34

SEASON 1961 1962 5 Cases nos. 15 17

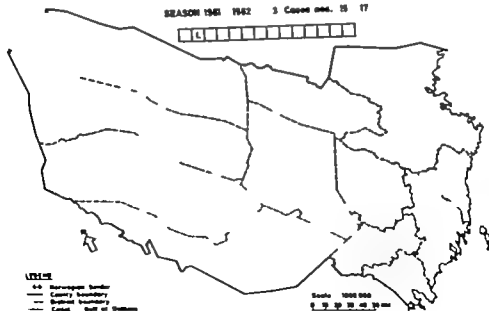


Fig 5 a; legend on page 34

The mean prevalence of EBN within each of the county's twelve municipal districts was found to vary from 0.02 to 0.21 cases per 1 000 inhabitants and year for the period studied - a ten-fold difference

The mean for the eight districts in the central northern and eastern parts of the county fell above or slightly below the county mean for the period examined; whereas for the four districts in the southern part of the county it fell well below that mean. For the seven districts comprising the inland region the mean prevalence of EBN for the period was 1.8 times higher than for the five districts comprising the coastal region (Fig 9 page 42 Appendix Table C)

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Domicile situated in the sparsely populated area of the county (x) in a built-up area other than town (o) or in a town (•)

SEASON 1984 1985. 2 Cases nos. 48 51

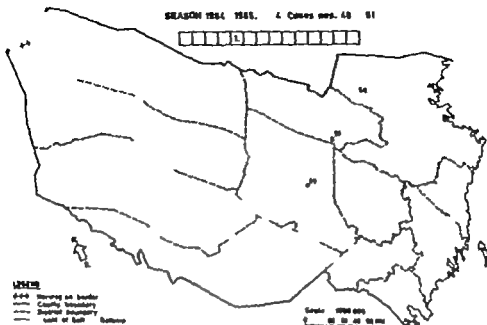


Fig 6 a; legend on page 34

SEASON 1985 1986 3 Cases nos. 52 60

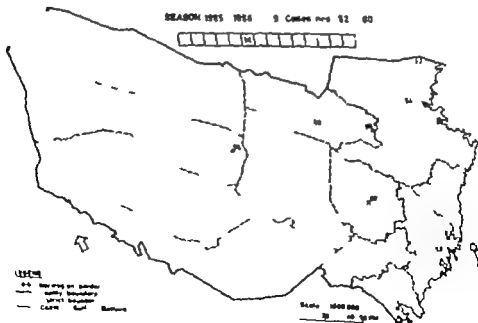


Fig 6 b; legend on page 34

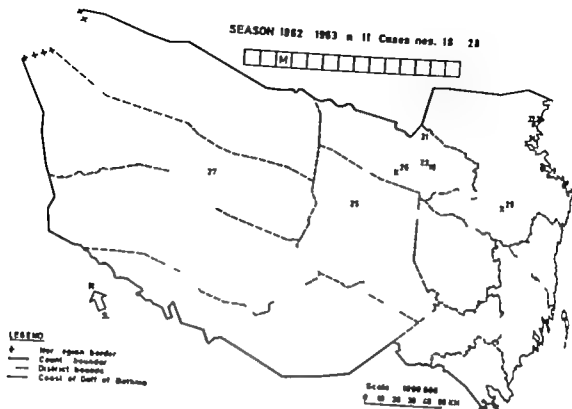


Fig 5 b; legend on page 34

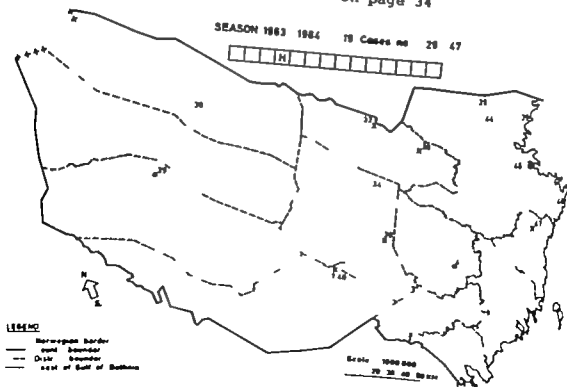


Fig 5 c; legend on page 34

SEASON 1961 1962. 4 Cases Nov 48 81

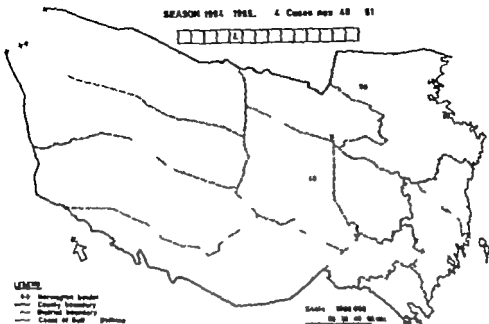


Fig 6 a; legend on page 34

SEASON 1965 1966. 9 Cases Nov 52 80

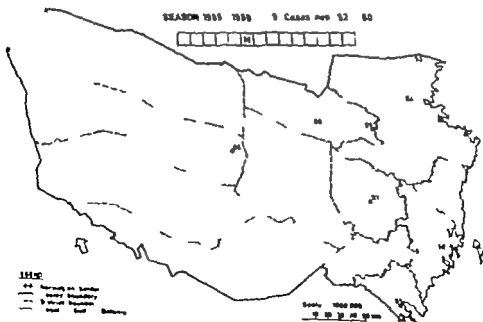


Fig 6 b; legend on

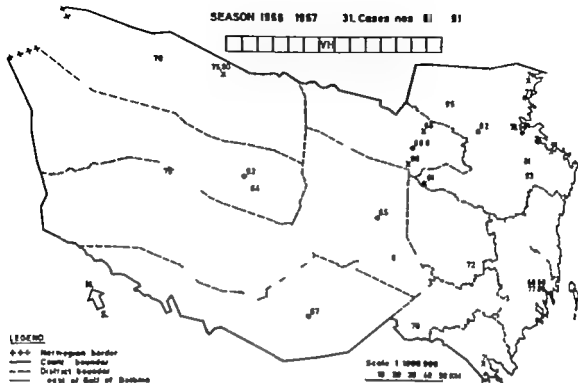


Fig 6 c; legend on page 34

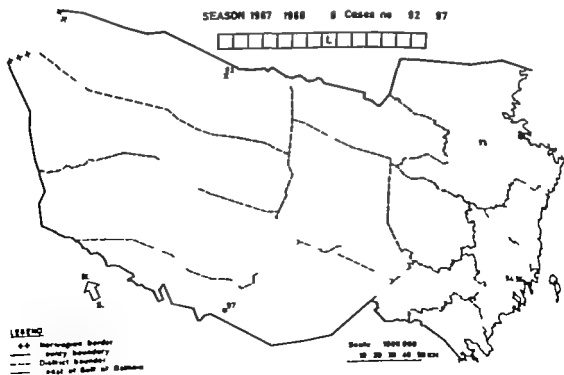


Fig 7 a; legend on page 34

SEASON 1968 1969. 7 Cases nos. 88 104

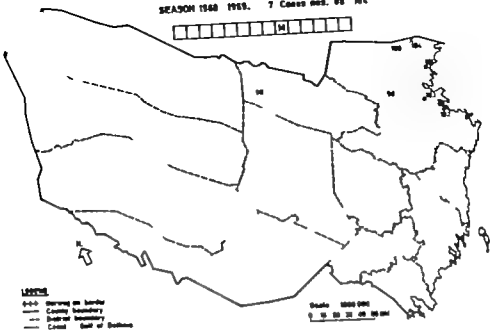
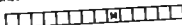


Fig 7 b; legend on page 34

SEASON 1969 1970 21 Cases nos 105 125

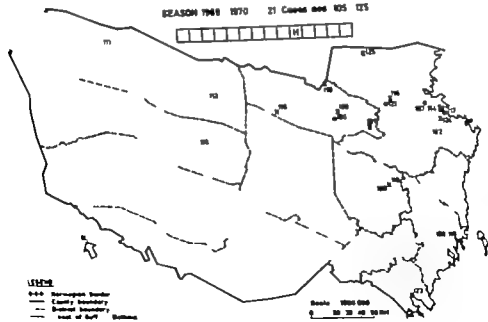


Fig 7 c; legend on page 34

SEASON 1972 1973 77 Cases max 196 173

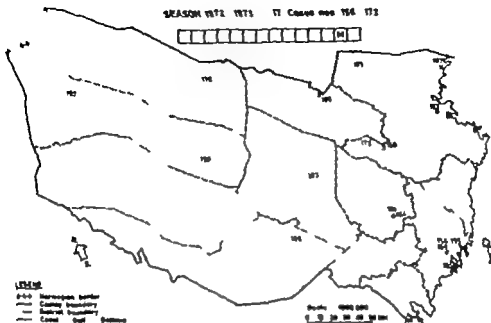


Fig. 8 b; legend on page 34

SEASON 1973 1974 95 Cases max. 173 207

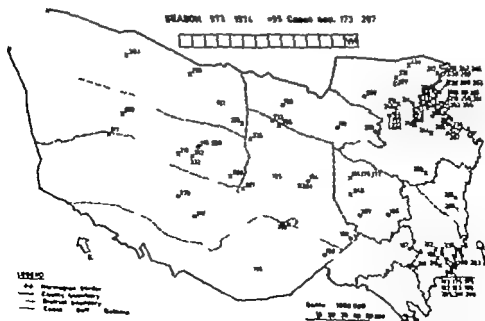


Fig. 8 c; legend on page 34

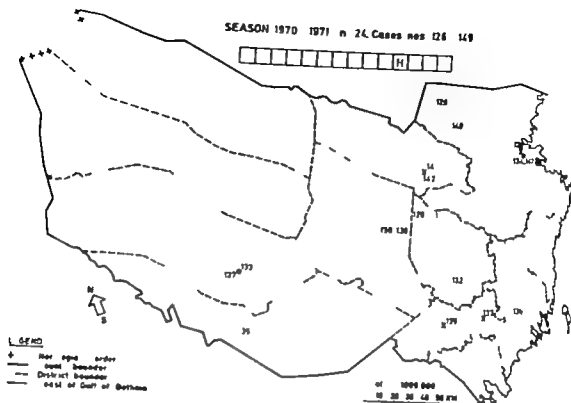


Fig 7 d; legend on page 34

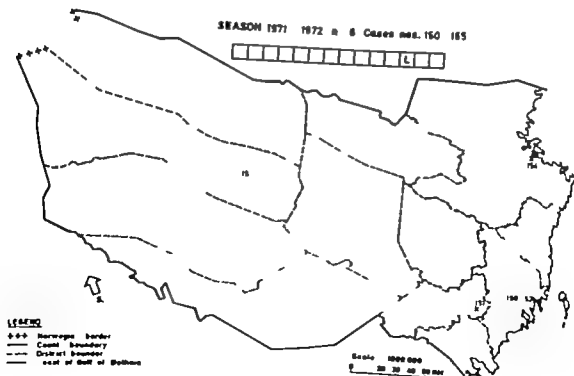


Fig 8 a; legend on page 34

III 5 Incidence of EBN

III 5 1 Monthly incidence

Table XI (page 44) shows the incidence (nos. of cases registered) of EBN in AC county for the period 1959 - 1975

The median incidence of EBN as observed in AC county was two cases per month for the period studied. The probability of the observed variation in monthly incidence being random was very small ($\chi^2 = 5.83$; $p < 0.001$); the hypothesis was tested by analyzing the sequence of events obtained when studying to what extent the number of cases registered each month (Table XI page 44) fell above or below the median. A run test (13)

III 5 2 Quarterly incidence

18.0 per cent of the cases of EBN were diagnosed during the first 10.8 per cent during the second 30.5 per cent during the third and 40.7 per cent during the fourth quarter of the year.

III 5 3 Yearly incidence

Cases of EBN were diagnosed for every year of the period studied but in a varying number ranging from two cases in 1959 and 1966 to 92 in 1974.

III 5 4 Seasonal incidence

Subsequently the incidence of EBN in AC county was studied for seasons each lasting from May one year to the following April i.e. for periods identical to those for which information on prevalence of small rodents in northern Sweden was presented in this study (section II 4 and III 6).

The results obtained when surveying the incidence of EBN in AC county in this way are presented in Fig. 10 (page 42).

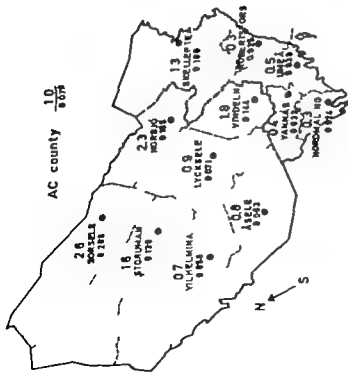


Fig. 9 Mean prevalence of EBN December 1959 - April 1974 for the 12 municipal districts of AC county and for the inland and the coastal region (figure below the name of the district/region); number of cases per 1 000 inhabitants and year. Above the name of each district/region is given the ratio of its mean prevalence to that of the county. Boundaries between municipal districts - - - and between inland and coastal regions

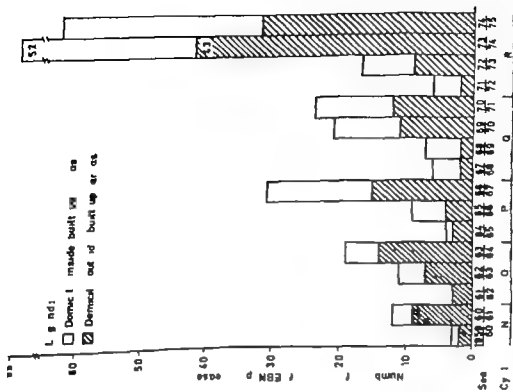


Fig. 10 Incidence of EBN in AC county for 16 consecutive seasons lasting from May one year to the following April. For designation of cycles cf the text

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Table XI Monthly incidence of EBM in AC county, Sweden, 1959 to 1975

Month	Year																	Total for	
	1959	-60	-61	-62	-63	-64	-65	-66	-67	-68	-69	-70	-71	-72	-73	-74	-75	month	quarter
Jan	1	2					1		2	3			5	14	1			29	
Feb		1							1		1			7				12	
March		1				2		2		1	1			4	7	1		19	60
April			1		1	1	1	1	1	1	1			2	3			12	
May				2	1			1			1			4	5			14	
June							1	1			2			3	3			10	36
July				3	1	1	4	2		1	4			4	19	1		40	
Aug		2	2	3			3	1		1	8			1	4	12	1	38	
Sept	1			1	2		2			1	2	2		8	4	1		24	102
Oct		1		1	1		8			1	7	2	1	1	10	7		40	
Nov		2		6	2		2	7		1		3	1	1	17	5		47	
Dec		1	5	2	3	1	4	3	1		6		2	1	14	6		49	136
Total	2	9	6	12	16	7	7	30	9	2	21	27	8	4	77	92	5	334	334

Subsequently any numerical difference in number of cases between seasons was rated as representing an interseasonal variation in the incidence of the disease. The main arguments for adopting this view were as follows

(a) The clinical records among which cases of EBN were sought were collected from the files of the three hospitals to which were referred for the period studied members of the entire county population in need of hospital care

(b) Subdividing the period examined into sections lasting from May one year to the following April does not alter the orderly sequence in which the cases were registered; therefore no obvious reason was found to assume that this procedure would appreciably influence the findings presented in section III 5 1 of this study viz a very low probability for the variation in number of cases of EBN being the consequence of chance variation

(c) Significant differences were found between the distributions of case of EBN for seasons with a separate grade of prevalence of small rodents (low middle high or very high); cf section III 7 3 page 51

The number of cases was found to increase for two to four consecutive seasons after which it dropped to rise again (Fig 10 page 42); this was the case for patients living outside (except for 1968/69) as well as inside built-up areas (except for 1963/64). Starting when a minimized incidence of EBN was recorded i.e. in the seasons of 1961/62 64/65 67/68 and that of 1971/72 the time interval studied covers five periods during which the seasonal incidence of EBN in AC county shifts in a similar fashion first rising and then falling

To test for the presence of any periodicity among the set of numbers representing the seasonal incidence of EBN as observed in AC county the interseasonal change in number of cases was compared at intervals of increasing length. This was done in the following way. The set of numbers representing the seasonal incidence of the disease was repeated below itself starting one (two three 14) season(s) after the first one examined. The change in number of cases between seasons (an increase or a decrease) was indicated separately in each row of number using arrows (cf Fig 11 page 50). The congruence of

Table XI Monthly Incidence of EBN in AC county Sweden; 1959 to 1975

Month	Year																	Total for	
	1959	-60	-61	-62	-63	-64	-65	-66	-67	-68	-69	-70	-71	-72	-73	-74	-75	month	quarter
Jan		1	2				1			2	3			5	14	1		29	
Feb			1							1		1		2	7			12	
March		1				2		2		1	1			4	7	1		19	60
April			1		1	1	1	1	1	1	1	1		2	3			12	
May					2	1		1				1		4	5			14	
June							1	1			2			3	3			10	36
July					3	1	1	4	2	1	4			4	19	1		40	
Aug			2	2	3		3	1		1	8		1	4	12	1		38	
Sept	1			1	2		2			1	2	2		8	4	1		24	102
Oct		1			1	1	8			1	7	1	1	1	10	7		40	
Nov		2		6	2		2	7		1		3	1	1	17	5		47	
Dec	1	5		2	3	1	4	3	1	6		2	1	14	6			49	136
Total	2	9	6	12	16	7	7	30	9	2	21	27	8	4	77	92	5	334	334

cycles (O - Q) two (O P) consisting of three and one (Q) of four seasons. The period studied ends with a cycle II composed of at least three seasons. Whether season 1974/75 constitutes the end of cycle R or the beginning of a new cycle (S) can not be decided yet.

From the season with the lowest incidence of EBN starting one cycle to the one with the highest incidence ending that cycle the mean number of cases rose 8.9 times (range 4.0-15.8). Cases of EBN were diagnosed for each of the 16 seasons covered in this study and in a rising number; during the first eight seasons 91 and during the eight last ones 239 cases were diagnosed a more than twofold increase. Also for seasons when a minimized incidence of EBN was recorded the number of cases doubled from three for season 1959/60 and 1961/62 to six for season 1967/68 and that of 1971/72.

XII 6 Seasonal prevalence of small rodents in northern Sweden

Table XII (page 48) presents information on the seasonal prevalence of small rodents in northern Sweden including AC county for the period 1959 - 1975 as reported by zoologists (section II 4.2). When summarizing the ratings of different observers information on the seasonal prevalence of animals was obtained for every season of the period studied.

For 13 of the 16 seasons studied numerical information was available in the form of catch statistic (14 - 16 - 22) or the extent to which rodents were found in stomachs of the red fox (10) indicating the change (increase or decrease) taking place between seasons in amount of small rodents captured or consumed as prey. When matching to individual seasons the numerical information available to the ratings of the small rodent prevalence presented an increase in the amount of animals between seasons was found to correspond to a rating of the prevalence as middle or high/very high (peak) and a decrease to a rating of the prevalence as low (Table XII page 48).

When examining the season sequentially the prevalence of small rodents was found to increase for a period of two and in one instance three consecutive seasons to reach high or very high levels during the following third or fourth season. This

changes in seasonal incidence of the disease was studied between rows by comparing to what extent corresponding arrows pointed in the same direction. This was done for each position of the two sets of numbers relative to each other obtained when increasing the distance between the opening seasons one season at a time. The results were tested statistically from the first to the ninth position applying a one-sided hypothesis and using a sign test (51).

When the interval between the sets of numbers amounted to three seasons (Fig 11 page 50) the interseasonal change in number of cases was found to coincide in 10 out of 12 instances where comparison between rows was possible; this result was significant when tested statistically ($p < 0.019$). For other positions i.e. a distance between opening seasons shorter or longer than three seasons the interseasonal changes in number of cases did not coincide to such an extent that significant results were obtained when statistically testing them; for distances longer than nine seasons the number of occasions when comparison between rows was possible diminished to such low numbers that statistical analysis using a sign test became inapplicable. These results were interpreted as follows:

When registered for periods from May one year to the following April the seasonal incidence of EBN in AC county was found to vary with such regular periodicity that its designation as a cyclical event was found appropriate. A successive increase in number of cases for three consecutive seasons was found to be the most common event; only one complete four-year cycle was observed.

A cyclical fluctuation in the seasonal incidence of the disease with a period of usually three but also four years was thus found to be a characteristic of EBN for the period and area studied.

Those seasons forming the interval between periods when a minimized number of cases of EBN was recorded are hereafter called a cycle and each of these intervals is designated with a letter (N - R). The period examined in this study starts with a period N (season 1959/60 and that of 1960/61) that probably constitutes the end of such a cycle. Then follow three complete

cycles (O - Q) two (O P) consisting of three and one (Q) of four seasons. The period studied ends with a cycle R composed of at least three seasons. Whether season 1974/75 constitutes the end of cycle R or the beginning of a new cycle (S) can not be decided yet.

From the season with the lowest incidence of EBM starting a cycle to the one with the highest incidence ending that cycle the mean number of cases rose 8.9 times (range 4.0-15.8). Cases of EBM were diagnosed for each of the 16 seasons covered in this study and in a rising number; during the first eight seasons 92 and during the eight last ones 139 cases were diagnosed a more than twofold increase. Also for seasons when a minimized incidence of EBM was recorded the number of cases doubled from three for season 1959/60 and 1961/62 to six for season 1967/68 and that of 1971/72.

III 6 Seasonal prevalence of small rodents in northern Sweden

Table XII (page 48) presents information on the seasonal prevalence of small rodents in northern Sweden including AC county for the period 1939 - 1975 as reported by zoologists (section II 4.2). When summarizing the ratings of different observers information on the seasonal prevalence of animals was obtained for every season of the period studied.

For 13 of the 16 seasons studied numerical information was available in the form of catch statistics (14 16 22) or the extent to which rodents were found in stomachs of the red fox (18) indicating the change (increase or decrease) taking place between seasons in amount of small rodents captured or consumed as prey. When matching to individual seasons the numerical information available to the rating of the small rodent prevalence presented an increase in the amount of animals between seasons was found to correspond to a rating of the prevalence as middle = high/very high (peak) and a decrease to a rating of the prevalence as low (Table XII page 48).

When examining the seasons sequentially the prevalence of small rodents was found to increase for a period of two and in one instance three consecutive seasons to reach high or very high levels during the following third or fourth season. This

Table XII

Seasonal prevalence from May to the following April of small rodents in northern Sweden including AC county; spring 1959 to 1975 Information cumulated from six different sources

Underlined letters indicate seasons when numerical information is available - in the form of catch statistics (14 16 22) or the extent to which rodents were found in stomachs of the red fox (10)

Ratings of the seasonal prevalence is presented verbally; L - a low M - a middle P - population peak equivalent to H - a high or VH - a very high prevalence of animals

Numerical information (10 14 16 22) is presented as the change in the variable studied taking place between seasons I - increase D - decrease compared with adjacent seasons Beginning (and end) of an evolutionary cycle of small rodents (N - R) of the text

Season and corresponding prevalence of small rodents																	
Refer- ence	1959	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75
(16)	H	P	L	M	P	L	I/M	I/P	VH	D/L	I						
(17)		P			P			P									
(10)		I	D								I	I	P			P	
(26)		P	L	M	P	L		P	L								
(27)		P			P			P									
(14)																	
(58)																	
(22)						L	M	VH	L		H	M	H	L	M	VH	
Cumulated infor- mation	M	H	L	M	H	L	M	VH	L		M	H	H	L	M	VH	L
Cycle	N				O				P				Q				R

sequence of events was repeated five times during the period studied and such a sequence is hereafter referred to as an evolutionary cycle of small rodents each of which is designated with a letter (N - R)

The period studied starts with two seasons (1959/60 and 1960/61) that possibly represent the conclusion of an evolutionary cycle of small rodents (N). Then follow two cycles (O P) consisting of three seasons each and one cycle (Q) composed of four seasons. The period studied ends with a season (1974/75) that might either form the end of cycle R or the beginning of another (S); the probability that it will form part of a five-year cycle can not be excluded however

III 7 Comparison between the incidence of EBM in AC county and the prevalence of small rodents in northern Sweden

III 7 1 Seasonal variations

In Fig 12 (page 50) variations in seasonal incidence of EBM in AC county (section III 5 4) were compared to correspondent changes in prevalence of small rodents in northern Sweden (section III 6) using arrow to indicate the change (a rise or fall) that took place between consecutive seasons in number of cases and in quantity of animals respectively

Using this method of comparison congruence was found for at least 14 and possibly 15 seasons in succession between interseasonal changes in prevalence of small rodents in northern Sweden and analogous variations in seasonal incidence of EBM in AC county which forms part of that territory. The probability that this outcome might be the consequence of chance variation is small ($p < 0.001$ using a sign test); the confidence of this result is influenced however also by the circumstance that for three of the seasons studied information on the prevalence of small rodents in northern Sweden was obtained solely asatings

Table XII

Seasonal prevalence from May to the following April of small rodents in northern Sweden including AC county; spring 1959 to 1975 Information cumulated from six different sources

Underlined letters indicate seasons when numerical information is available - in the form of catch statistics (14 16 22) or the extent to which rodents were found in stomachs of the red fox (10)

Ratings of the seasonal prevalence is presented verbally: L - a low M - a middle H - population peak equivalent to H - a high or VH - a very high prevalence of animals

Numerical information (10 14 16 22) is presented as the change in the variable studied taking place between seasons: I - increase D - decrease compared with adjacent seasons | Beginning (and end) of an evolutionary cycle of small rodents (N - R); of the text

Reference	Season and corresponding prevalence of small rodents															
	1959	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74
(16)	N	P	L	M	P	L	I/M	I/P	VH	D/L	I					
(17)		P			P			P				P				
(10)		I	D								I	I				P
(26)		P	L	M	P	L		P	L			P	P			
(27)		P			P			P				P	P			
(14)												P	P			P
(58)											I	I				
(22)						L	M	VH	L	M	H	H	L	M	M	VH
Cumulated information	M	H	L	M	H	L	M	VH	L	M	H	H	L	M	M	VH
Cycle	N						P				Q					R

III 7 3 Seasonal accumulation of cases

The registered interseasonal differences in number of cases of EBN were analyzed by summarizing the monthly number of cases separately for seasons (May - April) with a different grade of small rodent prevalence (low middle high or very high) The monthly totals obtained were then cumulated and the proportion each of them amounted to of the seasonal total was calculated Three cumulative frequency distributions at intervals of one month were thus obtained one for each type of season studied (cf Fig 18 page 55) The monthly proportions were then subtracted and the numerically largest difference (D) obtained was studied A two-sided hypothesis was tested and applying KOLMOGOROV SMIRNOV's two-sample test (51) the three differences studied were all found to be significant (Table XIII)

Table XIII Monthly proportion of cases of EBN registered in AC county for seasons (May - April) with a separate grade of prevalence of small rodents in northern Sweden: (L) low (M) middle (H) high or very high; (D) maximum difference between cumulated distributions A two-sided hypothesis was tested using KOLMOGOROV SMIRNOV's two-sample test P value denotes the probability that the two distributions compared have been drawn from the same population or from populations with the same distribution (Cf also Fig 18 page 55)

Seasons compared	D_{\max} of		Statistic	P
	$S_{n1}(X)$	$S_{n2}(X)$		
$S_{62}^{(L)} - S_{47}^{(M)}$	0 598		0 358	< 0 001
$S_{11}^{(L)} - S_{202}^{(H)}$	0 347		0 255	0 001
$S_{202}^{(H)} - S_{47}^{(H)}$	0 285		0 280	< 0 005

III 7 2 Cyclic variations

The seasonal changes in prevalence of animals characteristic of an evolutionary cycle of small rodents consist in a successive rise in quantity of animals terminated by a (usually) sharp fall in their number (section II 4) The seasonal incidence of EBN in AC county varied in a similar fashion (section III 5 4) These cyclic variations in seasonal incidence of EBN in AC county northern Sweden were found for the period studied to occur simultaneously and in phase with the cyclic variations in the seasonal prevalence of small rodents taking place in that area (section III 6)



Fig 11 Consecutive interseasonal (May to following April) changes (Δ increase ∇ decrease) in registered number of cases of EBN (figures within arrows) in AC county 1959 - 1975 compared at an interval of three seasons (cf the text) Interseasonal changes between rows (direction of correspondent arrows) concordant: + discordant: - $P < 0.019$ (sign test)

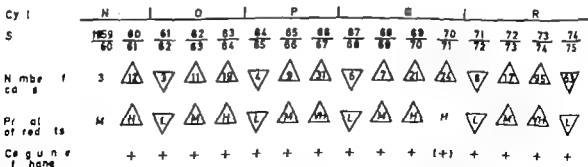


Fig 12 Consecutive interseasonal (May to following April) changes (Δ increase ∇ decrease) in number of cases of EBN observed in AC county 1959 - 1975 matched to changes in prevalence of small rodents in the area for correspondent periods Beginning (and end) of cycles (N - R) is indicated Interseasonal changes between rows (direction of correspondent arrows) concordant: + possibly concordant (+) $P < 0.001$ (sign test)

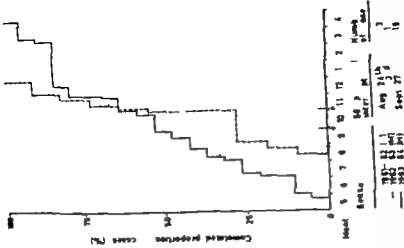


Fig 13
Cycle 0

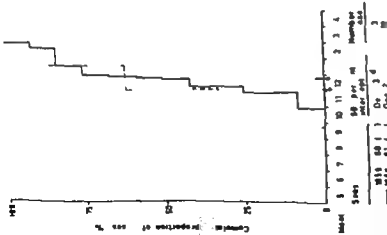


Fig 14
Cycle 0

Fig. 13-14 Cumulated proportions of cases of EHM diagnosed in AC county during seasons with operate grade of prevalence of small rodents in northern Sweden: (L) low (H) middle (H) high or (VII) very high The distributions obtained for seasons composing one evolutionary cycle of small rodents are displayed together on a common time scale The projection on the time scale of the 50th percentile intercept of each distribution examined is indicated with an arrow and the date thus obtained is given for each season studied

The very low probability that the differences analyzed might be the consequence of chance variation strongly supports the conclusion that the number of cases of EBN registered during seasons with a separate grade of prevalence of small rodents form distributions differing with respect to each other

The distribution of cases of EBN for seasons with a separate grade of prevalence of small rodents are drawn in Figs 13-17 and summarized in Fig 18 pages 53-55 To obtain these distributions the registered number of cases were summarized at three-day intervals and cumulated separately for each season studied on a time scale common to those seasons composing one evolutionary cycle of small rodents The projection on the time scale of the 50th percentile intercept was recorded separately for each season studied

When read as the intercept with the 50th percentile the mean interval between distributions obtained for seasons with a low and the following ones with a middle prevalence of small rodents was four months (range 2 3-5 6) The distributions obtained for seasons when the prevalence of small rodents was of a high or very high grade fell somewhere in between those obtained for the other seasons of the cycle when the prevalence of animals was of a different grade (low or middle) This was also the case for cycle II (Fig 16 page 54) consisting of two seasons with a prevalence of small rodents of a high grade in succession (that of 1969/70 and 1970/71)

The mean interval between seasons with a middle and those with a high or very high prevalence of small rodents was 1 5 months (range 0 7-2 4) when read as the intercept with the 50th percentile

77 per cent of the cases of EBN diagnosed when the prevalence of small rodents was of a low grade were registered during the first half (May - October) of these seasons When the prevalence of small rodents was of a middle grade the corresponding figure was 17 per cent and when the prevalence was of a high or very high grade it was 46 per cent

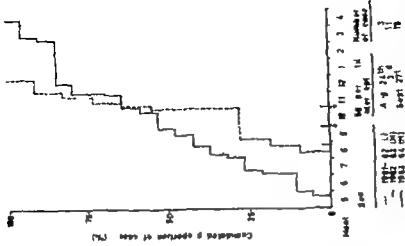


Fig 13
Cycle 8

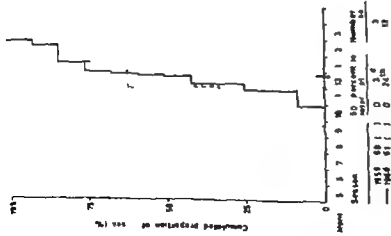


Fig 14
Cycle 8

Fig 13 18 Cumulated proportions of cases of EBW diagnosed in AC county during seasons with a parate grades of prevalence of small rodents in northern Sweden (L) low (M) middle (H) high or (VH) very high. The distributions obtained for seasons composing one evolutionary cycle of small rodents are displayed together on a common time scale. The projection on the time scale of the 50th percentile intercept of each distribution examined is indicated with an arrow and the date thus obtained is given for each season studied.

The very low probability that the differences analyzed might be the consequence of chance variation strongly supports the conclusion that the number of cases of EBN registered during seasons with a separate grade of prevalence of small rodents form distributions differing with respect to each other

The distribution of cases of EBN for seasons with a separate grade of prevalence of small rodents are drawn in Figs 13-17 and summarized in Fig 18 pages 53-55 To obtain these distributions the registered number of cases were summarized at three-day intervals and cumulated separately for each season studied on a time scale common to those seasons composing one evolutionary cycle of small rodents The projection on the time scale of the 50th percentile intercept was recorded separately for each season studied

When read as the intercept with the 50th percentile the mean interval between distributions obtained for seasons with a low and the following ones with a middle prevalence of small rodents was four months (range 2 3-5 6) The distributions obtained for seasons when the prevalence of small rodents was of a high or very high grade fell somewhere in between those obtained for the other seasons of the cycle when the prevalence of animals was of a different grade (low or middle) This was also the case for cycle Q (Fig 16 page 54) consisting of two seasons with a prevalence of small rodents of a high grade in succession (that of 1969/70 and 1970/71)

The mean interval between seasons with a middle and those with a high or very high prevalence of small rodents was 1 5 months (range 7-2 4) when read as the intercept with the 50th percentile

77 per cent of the cases of EBN diagnosed when the prevalence of small rodents was of a low grade were registered during the first half (May - October) of these seasons When the prevalence of small rodents was of a middle grade the corresponding figure was 17 per cent and when the prevalence was of a high or very high grade it was 46 per cent

Fig 17
Cycle R

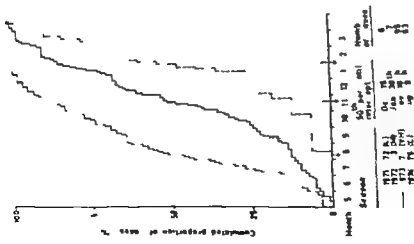
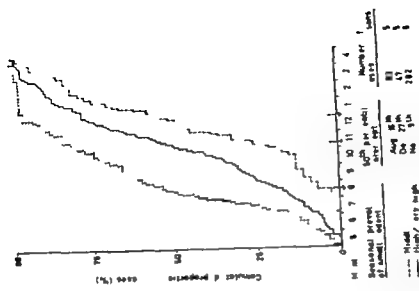


Fig 28
Composite picture showing the mean distribution for all cases of ZBM diagnosed for seasons with a separate grade of prevalence of small rodent during the period studied



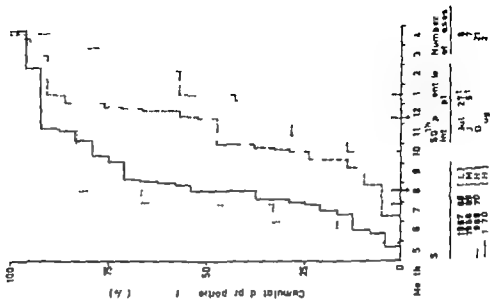


Fig 16
Cycle 16

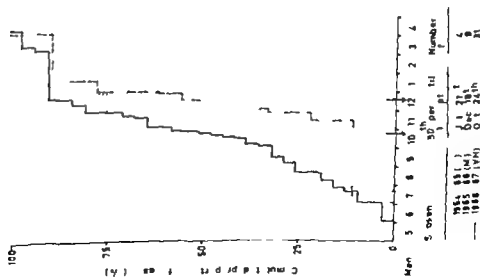


Fig 15
Cycle 15

(e) A three-year cycle is thus terminated by three seasonal halves in succession (the second half of the season with a middle and the two halves of the season with a high or very high prevalence of small rodents) i.e. a period of one year and a half characterized by an augmented incidence of EBN; 73 per cent ($n = 241$) of the total number of cases of EBN diagnosed during the period examined in this study were registered in that interval

(f) 19 per cent ($n = 63$) of the total number of cases of EBN diagnosed during the period examined in this study were registered during the first half of seasons when the prevalence of small rodents was rated as low

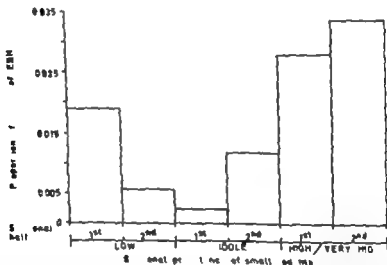


Fig 19 Proportion of cases of EBN registered in AC county for first (May - October) and second (November - April) halves of season with a separate grade of prevalence of small rodents in northern Sweden: low middle high or very high

III : Incidence of EBN in relation to meteorological variable

The actual values of the meteorological data collected were studied as well as their relative change between different years and for various intervals (months periods of the year) No consistent congruence could be discerned when the meteorological data were matched with the corresponding incidence of EBN; therefore the analysis was not carried out.

III 7 4 Survey of incidence of EBN in relation to prevalence of small rodents

When related to the changes in abundance of small rodents observed in northern Sweden the seasonal incidence of EBN in AC county was found to vary in a cyclical fashion with a periodicity of three to four years (section III 5 4) Within each cycle the following characteristics were observed regarding the appearance of cases of EBN (Fig 19 page 57)

(a) For seasons opening the cycle when the amount of small rodents was decreasing i e when the prevalence of animals was rated as low when compared with adjacent seasons the number of cases of EBN also decreased and the majority of cases diagnosed were registered during the first half (May - October) of these seasons

(b) For seasons when the amount of small rodents increased to reach an abundance rated as lying between that of the preceding and the following season(s) i e for seasons with a middle prevalence of small rodents the number of cases of EBN also increased to a level above that of the preceding season but not reaching the level of the following season and the majority of the cases diagnosed were then registered during the second half (November - April) of these seasons

(c) This accumulation of cases of EBN to the first seasonal half for seasons opening a cycle immediately followed by a season when the cases accumulate during the second seasonal half leaves a period of one year extending approximately from low-seasonal November to the following middle-seasonal October when comparatively few cases of EBN were observed; only eight per cent (n = 27) of the total number of cases diagnosed during the period examined in this study was registered in that interval

(d) For seasons when the amount of small rodents rose to the highest level during the cycle i e during seasons closing the cycle with a high or very high prevalence of small rodents the maximum number of cases of EBN was also reached and the cases were fairly evenly distributed over the two halves of these seasons

clinical entity separable from other conditions with an acute onset and accompanied by signs of kidney affection; this view was also adopted in this study. Probably subclinical cases exist however where the disease runs a course mild enough not to force the patients to seek medical advice.

All patients matching the applied criteria were accepted as cases of EBN; only a few probably less than ten were found who had an original diagnosis other than epidemic nephropathy or EBN. The day of falling ill was estimated for all cases without difficulty.

Although care was taken to apply the diagnostic criteria uniformly throughout the whole study the probability remains of erroneous decisions having been made when assessing the information obtained from the clinical records. Moreover cases of EBN might also have been missed if a because the bulk of the record examined had to be kept within reasonable limits. The magnitude of the error thus introduced into this study cannot be estimated with certainty but is rated to be small enough not to influence appreciably the main results presented; one reason for this conclusion is the cyclic variation demonstrated for the seasonal incidence of the disease.

The incubation time of EBN and its mode of transmission still being undetermined no effort was made in this study to seek information on the patients' activities prior to contracting the disease. The majority of patients reported having been at home when the illness began and therefore the sites of their domiciles were examined when studying the prevalence of the disease.

IV 2 Distribution of cases among different sections of the county population

51.1 per cent of the mean population in AC county for the period studied consisted of males. Among these 77.3 per cent of the cases of EBN were diagnosed a proportion close to that reported from Finland 80.3 per cent (25) but lower than that obtained in a previous investigation carried out in AC county (45) where 88.9 per cent of the patients were men.

In this study the male predominance was demonstrated for

IV DISCUSSION

IV 1 Design of the present study

The present study was limited to AC county the area estimated to be sufficiently large for cases of EBN if any to be encountered even during years with very low incidence of the disease

January 1959 was chosen as the starting point because clinical records written before that date generally were rated as containing an insufficient amount of information to meet the standards set up for this study. The period thus covered was estimated to be of a length sufficient to yield enough material to fulfil the purpose of this study.

The discriminating characteristics applied in this study when documenting cases of EBN were set up using information from papers on epidemic nephropathy containing a many detailed casuistical reports; in addition personal experience had been gained of cases seen at Umeå University hospital; the criteria had to be applicable also to records written in county hospitals using standard diagnostic procedures (Skellefteå and Lycksele).

The case histories obtained were all quite similar and in essence identical with those repeatedly summarized in the literature on epidemic nephropathy. Characteristically the patients were suddenly taken ill with a rapidly rising body temperature for which no obvious cause could be demonstrated. The condition was accompanied by a distressing ache and pain mostly in the back and in the abdomen to which was added lack of appetite, nausea, vomiting, obstipation and a feeling of fullness; sometimes however diarrhoea was present. Proteinuria and elevated concentration of serum non protein nitrogen were found in almost all cases studied. The disease was self-limiting; full recovery was the rule and the course was uneventful for all cases examined in this study.

In spite of the repeated inability to demonstrate an etiologic agent capable of generating the symptoms and signs in these patients, most observers agree that the disease is a

clinical entity separable from other conditions with an acute onset and accompanied by signs of kidney affection; this view was also adopted in this study. Probably subclinical cases exist however where the disease runs a course mild enough not to force the patients to seek medical advice.

All patients matching the applied criteria were accepted as cases of EBN; only a few probably less than ten were found who had an original diagnosis other than epidemic nephropathy or EBN. The day of falling ill was estimated for all cases without difficulty.

Although care was taken to apply the diagnostic criteria uniformly throughout the whole study the probability remains of erroneous decisions having been made when assessing the information obtained from the clinical records. Moreover cases of EBN might also have been missed, i.e. because the bulk of the records examined had to be kept within reasonable limits. The magnitude of the error thus introduced into this study cannot be estimated with certainty but is rated to be small enough not to influence appreciably the main results presented; one reason for this conclusion is the cyclic variation demonstrated for the seasonal incidence of the disease.

The incubation time of EBN and its mode of transmission still being undetermined no effort was made in this study to seek information on the patients' activities prior to contracting the disease. The majority of patients reported having been at home when the illness began and therefore the sites of their domiciles were examined when studying the prevalence of the disease.

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51.1 per cent of the mean population in AC county for the period studied consisted of male. Among these 77.5 per cent of the cases of EBN were diagnosed, a proportion close to that reported from Finland, 80.3 per cent (29) but lower than that obtained in a previous investigation carried out in AC county (45) where 89.9 per cent of the patients were men.

In this study the male predominance was demonstrated for all age-groups studied.

IV DISCUSSION

IV 1 Design of the present study

The present study was limited to AC county the area estimated to be sufficiently large for cases of EBN if any to be encountered even during years with very low incidence of the disease

January 1959 was chosen as the starting point because clinical records written before that date generally were rated as containing an insufficient amount of information to meet the standards set up for this study The period thus covered was estimated to be of a length sufficient to yield enough material to fulfil the purpose of this study

The discriminating characteristics applied in this study when documenting cases of EBN were set up using information from papers on epidemic nephropathy containing a many detailed casuistical reports; in addition personal experience had been gained of cases seen at Umeå University hospital; the criteria had to be applicable also to records written in county hospitals using standard diagnostic procedures (Skellefteå and Lycksele)

The case histories obtained were all quite similar and in essence identical with those repeatedly summarized in the literature on epidemic nephropathy. Characteristically the patients were suddenly taken ill with a rapidly rising body temperature for which no obvious cause could be demonstrated. The condition was accompanied by a distressing ache and pain mostly in the back and in the abdomen to which was added lack of appetite, nausea, vomiting, obstipation and a feeling of fullness; sometimes however diarrhoea was present. Proteinuria and elevated concentration of serum non protein nitrogen were found in almost all cases studied. The disease was self-limiting; full recovery was the rule and the course was uneventful for all cases examined in this study

In spite of the repeated inability to demonstrate an etiologic agent capable of generating the symptoms and signs in these patients; most observers agree that the disease is a

from AC county (45-47) where 66.7 per cent of the cases were of that age but lower than the proportion reported in a Norwegian study (61) 78.6 per cent. In a study covering the whole of Finland (29) 57.8 per cent of the cases were found among people aged 20-40 years.

Seven of the patients were 15 years or younger. This proportion 2.6 per cent is the lowest reported among children. In a study from Norway (61) 3.6 per cent of the cases were children and the corresponding proportion in previous studies carried out in AC county was 5.5 per cent (45) and 11.5 per cent (47) respectively. In a Finnish study (29) the overall proportion of children was 5.7 per cent but in the south-eastern part of the country it was as high as 13 per cent being the highest reported proportion of cases among non-adults. The low proportion of children among the cases of EBN examined in this study could not be satisfactorily explained but the children were not studied separately mainly because of the few cases diagnosed among them. No pediatric study on cases of EBN has hitherto been published and as the diagnostic criteria applied in this study were chosen from findings made in adults they are possibly not directly applicable to children.

IV 3 Changes in geographic distribution of county population for the period studied

For years other than 1960, 1965 and 1970 when a census was carried out the number of people living outside and inside built-up area respectively (Table III V pages 19, 21 and 23 respectively) had to be calculated. This was done linearly as the number of people inhabiting the sparsely populated area of the county was found to decrease at an almost constant rate whether studied for the inland or for the coastal region.

IV 4 Prevalence of EBN in areas of different population density

For the period studied a significantly higher prevalence of EBN (number of cases per 1 000 inhabitants and year) was found among that part of the county population inhabiting the sparsely populated area than among those living in the built-up area (section III 1). The gradual decline during the period

ulated area of the county and for those living in the built-up areas as well females 65 years or older in built-up areas being an exception. A satisfactory explanation for this male predominance among people diagnosed as cases of EBN could not be found in this study. Assuming EBN to be of an infectious origin a difference in susceptibility to the infectious agent might be assumed to exist between the sexes, males being more sensitive to it than females.

A difference between the sexes in maximum serum creatinine concentration and in age similar to that demonstrated in this study was found among 12 patients from Norway (31). Two-thirds of these cases were men with a mean age of 36 years and a mean maximum serum creatinine concentration of 5.8 mg per 100 ml. The corresponding figures for the female cases were 46 years and 6.9 mg per 100 ml respectively. No explanation was found in this study for these differences in maximum serum creatinine concentration between the sexes.

The occupational survey presented in Table X (page 31) indicates that cases of EBN were diagnosed among people of very different professions and among different social classes of the community. Previous reports from Sweden in 1963 (45) and Norway in 1954 (61) that the majority of the cases with nephropathy were to be found among certain occupational classes, mostly farmers and/or lumberers, were not substantiated by the results of this study. The report from Finland in 1971 (29) that 70 per cent of the patients were farmers or members of farm families could not be verified among the cases from AC county.

The number of people occupied in forestry and farming has declined substantially in Sweden during the last decade, a fact that might explain partly the different proportions of cases among lumberers and farmers reported at different times and from different Scandinavian countries.

The morbidity caused by EBN among people occupied in medical service was not raised (section III 2.3) and no evidence was found that they had fallen ill as a consequence of attending cases of the disease.

71.5 per cent of the cases were aged 20-40 years, a proportion slightly higher than that reported in previous studies.

from AC county (45-47) where 66.7 per cent of the cases were of that age but lower than the proportion reported in a Norwegian study (61) 78.6 per cent. In a study covering the whole of Finland (29) 57.8 per cent of the cases were found among people aged 20-40 years.

Seven of the patients were 15 years or younger. This proportion 2.1 per cent is the lowest reported among children. In a study from Norway (61) 3.6 per cent of the cases were children and the corresponding proportion in previous studies carried out in AC county was 5.5 per cent (45) and 11.5 per cent (47) respectively. In a Finnish study (29) the overall proportion of children was 5.7 per cent but in the south-eastern part of the country it was as high as 13 per cent being the highest reported proportion of cases among non-adults. The low proportion of children among the cases of EBH examined in this study could not be satisfactorily explained but the children were not studied separately mainly because of the few cases diagnosed among them. No pediatric study on case of EBH has hitherto been published and as the diagnostic criteria applied in this study were chosen from findings made in adults they are possibly not directly applicable to children.

IV 3 Change in geographic distribution of county population for the period studied

For years other than 1960, 1965 and 1970 when a census was carried out the number of people living outside and inside built up areas respectively (Table III-V pages 19-21 and 23 respectively) had to be calculated. This was done linearly as the number of people inhabiting the sparsely populated area of the county was found to decrease at an almost constant rate whether studied for the inland or for the coastal region.

IV 4 Prevalence of EBH in areas of different population density

For the period studied a significantly higher prevalence of EBH (number of cases per 1 000 inhabitants and year) was found among that part of the county population inhabiting the sparsely populated area than among those living in the built-up areas (section III 1). The gradual decline during the period

studied in number of people living outside the built-up areas of the county was not accompanied by a proportionate lowering of the prevalence among that part of the county population nor by a proportionate increase in prevalence among the steadily growing population of the built-up areas; the prevalence of EBN continued to be higher among people living outside built-up areas than among those living inside these areas for the whole period studied. Consequently the relation in prevalence between the areas differing with respect to population density remained unaltered in spite of the substantial shift of the county population taking place during the period studied.

The prevalence of EBN was also significantly higher among the inhabitants of the thinly populated inland region than among people inhabiting the more densely populated coastal region.

Cases of EBN occurring among town-dwellers are mentioned only in passing by previous investigators (29) which may convey the impression that they might be rare. Such a view was not supported by the results of this study. 18.7 per cent of the cases being diagnosed among town-dwellers.

Between people living outside and those living inside built-up areas of the county no difference was found in the distribution of population or of cases according to sex and age-groups sufficiently large to explain the higher mean prevalence of EBN among that part of the county population living outside built-up areas.

No reason was found in this study to assume differences in hygienic conditions, standard of living or food habits to exist between the county population living inside and that living outside the built-up areas of a magnitude sufficient to cause discernible differences in susceptibility to a supposed infectious agent. Moreover, assuming the prevalence of EBN to be appreciably influenced by such factors, then the advance in social welfare made during the period covered by this study would most likely have resulted in a drop in the prevalence of the disease which was not the case. The prevalence of the disease was instead found to increase during the period studied and the highest prevalence registered in this study was for the last season examined in detail, that of 1973/74.

Sometimes the view is expressed that people living in rural surroundings or far from community centres seek medical advice proportionately less often than do people inhabiting built up areas. Such a mechanism might have been at work in the material examined in this study but its effect would then have been to diminish the proportion of cases diagnosed among that part of the population living outside built up areas. Therefore such a mechanism could not explain the higher prevalence found among people living in the sparsely populated area of the county.

Using the maximum serum creatinine concentration registered during the acute phase of the disease as a measure of the severity of the kidney affection sustained no significant difference was found when comparing the means for males living outside and inside built up areas nor for females inhabiting corresponding areas. Differences in the severity of the kidney reaction therefore did not explain the differences in prevalence of EBM found in this study between the county population living outside and inside built-up areas.

In this section are discussed those results of the present study that demonstrate a significantly higher prevalence of EBM to be present among people in AC county living more distantly apart than among those living more closely grouped using the concept population density in two different ways: population of the sparsely populated area of the county versus that of the built up areas and population of the thinly populated inland region versus that of the more densely populated coastal region. These results may convey the impression that an inverted relationship exists between the population density of AC county and the prevalence of EBM in that area; they might possibly be explained by assuming the existence of a contagious factor more prevailing outside than inside those parts of the county where people live closely grouped (towns and built up areas).

IV 5 Geographic distribution of cases

Cases of EBN diagnosed in AC county during the separate seasons examined in this study were found to vary considerably in number and also to appear within areas of very different sizes ranging from two of the 12 municipal districts of the county to the entire area studied (section III 4)

When observing the order in which the cases appeared and surveying the geographic positions of their domiciles relative to each other no definite starting point(s) for the (seasonal) outbreaks of the disease could be discerned within the area(s) examined . Especially during seasons when many cases of EBN were diagnosed it was evident that within a brief period they appeared in places widely apart and no evidence was obtained indicating that the disease was propagated from one place within AC county to another . Neither could any contact between the cases be demonstrated the four instances where two and three members of the same families respectively contracted the disease within a short interval being an exception . probably without real significance as they all occurred within the sparsely populated area of the county where the major proportion of the cases contracted the disease

When calculating the mean yearly prevalence for the whole period studied separately for each municipal district a figure equivalent to or higher than the county mean was obtained for those districts situated in the central and northern parts of the county and in the eastern parts of the inland region while the corresponding figure for the remaining parts of the county was below that of the county mean . The longitudinal difference between municipal districts of AC county in mean prevalence of cases of EBN for the period studied might possibly be a consequence of the tendency of field populations of small rodents to fluctuate with an augmented amplitude on more northerly latitudes than on more southerly ones (section II 4 1 c) . No explanation was found in this study for the difference observed in mean prevalence of EBN between eastern and western municipal districts of the inland region of AC county

IV 6 The endemic nature of the disease

In the area(s) examined and for the period studied no evidence could be demonstrated in favour of EBN a being of an epidemic nature in the sense of a (contagious) disease that might spread over large areas usually then affecting a considerable proportion of the population. From a nosographic and epidemiologic point of view the disease studied here is therefore better characterized as being of an endemic nature. The main arguments for this opinion are:

- (a) cases appeared anywhere in the area studied
- (b) cases were diagnosed every year during the period examined
- (c) the low prevalence
- (d) no evidence was obtained in favour of the disease being propagated from person to person

IV 7 Incidence of EBN in AC county

Cases of EBN were diagnosed in the area examined for every season studied. The seasonal incidence of the disease was found to change in a cyclical fashion although with variations in amplitude. No clue to the cause of these variations was found when examining the individual case histories. Neither were any variations found similar to those recorded for the seasonal incidence of EBN when studying the climatic conditions prevailing in the sea during the period covered by this study.

IV 5 Geographic distribution of cases

Cases of EBN diagnosed in AC county during the separate seasons examined in this study were found to vary considerably in number and also to appear within areas of very different sizes ranging from two of the 12 municipal districts of the county to the entire area studied (section III 4)

When observing the order in which the cases appeared and surveying the geographic positions of their domiciles relative to each other no definite starting point(s) for the (seasonal) outbreaks of the disease could be discerned within the area(s) examined Especially during seasons when many cases of EBN were diagnosed it was evident that within a brief period they appeared in places widely apart and no evidence was obtained indicating that the disease was propagated from one place within AC county to another Neither could any contact between the cases be demonstrated the four instances where two and three members of the same families respectively contracted the disease within a short interval being an exception probably without real significance as they all occurred within the sparsely populated area of the county where the major proportion of the cases contracted the disease

When calculating the mean yearly prevalence for the whole period studied separately for each municipal district a figure equivalent to or higher than the county mean was obtained for those districts situated in the central and northern parts of the county and in the eastern parts of the inland region while the corresponding figure for the remaining parts of the county was below that of the county mean The longitudinal difference between municipal districts of AC county in mean prevalence of cases of EBN for the period studied might possibly be a consequence of the tendency of field populations of small rodents to fluctuate with an augmented amplitude on more northerly latitudes than on more southerly ones (section II 4 l c) No explanation was found in this study for the difference observed in mean prevalence of EBN between eastern and western municipal districts of the inland region of AC county

IV 9 Associations between the incidence of EBN and the prevalence of small rodents

In this study the relationship between the incidence of EBN as observed in AC county and the prevalence of small rodents in northern Sweden was examined in different ways

(a) A close correspondence was found between the seasonal and cyclic variations of the two phenomena (section III 7 1 - 2)

(b) Chance was found to be an unlikely explanation for the variations observed in the incidence of the disease (section III 3 1) and when comparing the number of cases diagnosed during seasons with a separate grade of prevalence of small rodents the distributions obtained were found to differ significantly (section III 7 3)

(c) The findings in (b) were illustrated by the graphic pictures obtained when plotting the cumulated proportions of number of cases on a time scale common to seasons composing evolutionary cycles of small rodents (Figs 13-18 pages 53-55); the distributions for seasons differing with respect to prevalence of small rodents were found to run more or less spaced and to reach the 50th percentile at different times of the season(s)

(d) Also the proportion of cases diagnosed during first and second halves of seasons with a separate grade of prevalence of small rodents was found to differ (section III 7 4 ; Fig 19 page 57)

A tentative explanation for the findings in (c) and (d) might be found when relating them to changes in number of animal characteristic of an evolutionary cycle of small rodents (1 35)

During the first and possibly also during the second half of seasons opening an evolutionary cycle of small rodents the number of animals declines to (very) low numbers. The intercept with the 50th percentile for distributions obtained when cumulating the proportion of cases of EBN diagnosed during these seasons fell on an average in the ^{middle} ~~beginning~~ of September, i.e. in the first seasonal half during which 19 per cent of the total seasonal number of cases studied (n = 331) were registered

IV 8 Information on prevalence of small rodents in northern Sweden

The validity of the general outline of small rodent field population dynamics accounted for in section II 4 1 of this study will not be discussed in this presentation the topic being covered in literature on ecological zoology Likewise no attempt was made to appraise the reliability of each individual observer's ratings of the prevalence of small rodents including the numerical information presented Most observers referred to in this study use verbal descriptions when reporting the prevalence of small rodents Using information of this kind in the manner adopted in this study (section III 7) will inevitably introduce a factor of uncertainty The circumstance however that the majority of individual observers report similar estimations of the seasonal prevalence of small rodents in northern Sweden confers upon the information obtained a degree of reliability rated as sufficient for the purpose of this study

One of the consequences of describing the seasonal prevalence of small rodents verbally is that the terms used recur in an identical order within each evolutionary cycle examined Although repeated in this way (low middle high or very high - peak) verbally identical terms must not be interpreted as standing for an always identical quantity of small rodents; they are used expressly with the intent of characterizing the actual seasonal prevalence of animals compared to that of adjacent seasons Consequently they also indicate the change in quantity of animals estimated to have taken place between seasons (an increase or a decrease) except for seasons rated as having a similar prevalence of small rodents (that of 1969/70 and 1970/71)

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while the corresponding figure for the second seasonal half was six per cent

During the following season of the evolutionary cycle of small rodents the number of animals increases, also during (part of?) its second half and for these seasons the intercept with the 50th percentile fell on an average at the end of December i.e. in the second seasonal half for which 12 per cent of the total number of cases studied were observed. During the first seasonal half the corresponding figure was only two per cent the minimum proportion of cases registered for seasonal halves of the small rodent evolutionary cycle.

During seasons closing the cycle the number of animals continues to increase at least during the first half of these seasons to reach maximum levels during the cycle. For these seasons the intercept with the 50th percentile fell on an average in the beginning of November i.e. shortly after mid-season (October 31st). During the first half of these seasons 28 per cent of the total number of cases were registered and during the second half 33 per cent the latter figure being the maximum proportion of cases registered for seasonal halves of the small rodent evolutionary cycle.

These findings were interpreted as being in favour of an intraseasonal correspondence existing between the two phenomena studied possibly being associated with changes in the age distribution of animals during the evolutionary cycle of the small rodent population.

IV 10 Consistency of the main results of this study

The results presented in section III 7 of this study showing a close correspondence between the incidence of endemic benign nephropathy in AC county and the prevalence of small rodents in northern Sweden are consistent with the documentation presented in section III 1 of a higher prevalence of the disease among people living more distantly apart i.e. in areas that constitute biotopes favourable for small rodents than among those living more closely grouped i.e. in built-up areas or in towns.

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- 59 Tomasson B Landlæknir, Director general of Public
Health Reykjavik Iceland: Personal communication

Con secu- tive num- ber of case	Ini Occupation or civil state	Sex	Age	Plac of re- idence	Cate- gory	nu- mer- ical site	Date of onset of illness	Maximum req'd value of				Type of diure- tic	a ot- abnor- mal ma- lity Comments
								(j)	(k)	(l)	(m)		
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)	(n)
1	FK G	Tractor driver	M	29	BU	1	59 12 01		4		MPM 112	P	Shivers
2	WKAR	Builder & mate	M	40	SP	9	60 01 21	40	10	11 6	O/P		
3	SGL	National forest ranger	M	33	BU	9	10 28	38 2	0 5	2 0	NO		
4	EP-OL	Engineer	M	24	BP	5	11 26	> 40	5	5 9	P		
5	WGH	Workshop manager	M	21	BU	9	11 27	40 2	4 0	3 0	NI		
6	AKA	Tractor driver	M	26	BU	9	12 10	39 5	2 5	1 5	NI		
7	BEP	Road-grader driver	M	40	SP	9	12 10	40 2	+	2 6	P?		
8	DTSJ	Forest worker	M	24	SP	5	12 25	39 5	3	1 1	P		Appendectomy performed
9	CK E	Farm owner	M	36	SP	9	12 26	NEP	6	8 5	P		
10	LTD	Farm/forest worker	M	49	SP	9	12 31	NI	4	4 2	P		
11	QJA	Forest worker	M	16	SP	9	61 01 01	40	0 5	6 2	P		
12	LSM	Ore prospector	M	41	SP	5	01 23	39	5 5	4 8	P		
13	LTH	Farm worker	M	18	SP	9	02 25	38 2	+	MPM 65	NI		
14	LKO	Miner	M	30	SP	5	03 01	38 6	2 5	8 1	P		

Appendix Table A (pages 77-89) Demographic data for patients diagnosed as cases of endemic benign nephropathy in AC county northern Sweden December 1959 - April 1974 and some of the findings recorded during the acute phase of the disease

Notes

- (a) The patients are tabulated in chronological order according to date of onset of illness Beginning (May 1st) and end (April 30th) of seasons is indicated: - - - - -
- (d) M: male; F: female
- (e) Age in years on date of onset of illness
- (f) The place of residence is classified as being situated either in a sparsely populated area (SP) or in a built-up area (BU) including that of a town (T)
- (g) The municipal districts of AC county are designated as follows
 Within the inland region: 1 - Sorsele 2 - Storuman 3 - Vilhelmina 4 - Åsele 5 - Norra
 6 - Lycksele 7 - Vindel
 Within the coastal region: 8 - Vännäs 9 - Skellefteå 10 - Robertsfors 11 - Umeå
 12 - Nordmaling
- (j-l) Figures or symbols denote maximum value of parameter recorded or reported during the acute phase of the disease
- (j) C: Degrees Centigrade NE: Body temperature not elevated SE: slightly elevated
- (k) Figures denote g protein per 1 000 ml of urine Using a strip test TR denotes trace
 amount of protein in urine + an amount of protein roughly corresponding to 30 mg per 100
 ml of urine ++ to 100 +++ to 300 and ++++ to > 1 000 mg of protein per 100 ml of urine
 Neg: Proteinuria not observed
- (l) Figures denote creatinine concentration in mg per 100 ml of serum if not stated as NPN
 (non-protein nitrogen) or BUN (blood-urea nitrogen)
- (m) Degree of diuretic abnormality is stated as volume of urine per 24 hours during stay in
 hospital the urinary volume recorded on day of admission being excluded O - oliguria
 (< 400 ml) P - polyuria (> 3 000 ml) NO - no diuretic abnormality recorded
- NI No information available
- ? Information questionable
- Denotes reference to information given in (n)

Con- s cu- tive num- ber of cases	Ini- tial	Occup ation or civil state	Sex	Age	Place of re- sidence	cate- gory	Date of onset of illness	para- pro- tural urine OC	Maximum regis- tered value of			Type of diure- tic	
									(j)	(k)	(l)		(m)
(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	(k)	(l)	(m)	(n)
1	PK	Tractor driver	M	29	BU	1	59 12 01			4	MPW	P	Shivers
											112		
2	WTAR	Builder's mate	M	40	SP	9	60 01 21	40	10	11 6	O/P		
3	SQL	National fore t ranger	M	33	BU	9	10 28	38 2	0 5	2 0	NO		
4	EP-OL	Engineer	M	24	GP	5	11 26	> 40	5	5 9	P		
5	WSH	Workshop manager	M	21	DU	9	11 27	40 2	4 0	3 0	NI		
6	AXA	Tractor driver	M	26	BU	9	12 10	39 5	2 5	1 5	NI		
7	BEP	Road-grader driver	M	40	DE	9	12 10	40 2	+	2 6	P?		Appendectomy performed
8	BTSJ	Forest worker	M	24	SP	3	12 25	39 5	3	1 1	P		
9	GK-E	Farm owner	M	36	GP	9	12 26	NE?	6	6 5	P		
10	LTD	Farm/forest worker	M	49	SP	9	12 31	NI	4	4 2	P		
11	QJA	Forest worker	M	16	SP	9	61 01 01	40	0 5	6 2	P		
12	LSM	Ore prospector	M	41	SP	5	01 23	39	5 5	4 6	P		
13	LTH	Farm worker	M	18	SP	9	02 25	38 2	+	MPW	NI		
										65			
14	LKO	Miner	M	30	SP	5	03 01	38 6	2 5	8 1	P		

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
15	HAGA	Forest worker	M	14	SP	5	08 02	39 4	+	0 8	NO	
16	AG	Forest worker	M	36	SP	2	08 25	41	+++	NPN	P	
17	BUI	Domestic help	F	22	SP	9	62 04 13	39 4	T	0 9	NI	
18	BK-A	Forestry foreman	M	37	BU	5	08 13	39 5	3	2 6	O/P	
19	S-AO	Canteen managers	F	46	BU	9	08 25	40 4	+	1 1	NI	
20	NECA	Farm owner s/forest worker s wife	F	42	SP	9	09 06	39 9	4 5	6 1	O/P	
21	AKGO	Forest worker	M	28	SP	5	11 01	38 4	2	1 4	P	
22	NMK	Housewife	F	56	BU	9	11 02	39	4	1 2	NI	
23	DMA	Forest worker	M	41	SP	5	11 04	38 8	2 5	2 1	NO	
24	EBK	Cook s assistant	F	36	SP	9	11 09	>38 6	+	3 8	P	
25	HAE	Manager	M	48	SP	6	11 15	39	3 5	6 6	O/P	
26	BTW	Excavator	M	34	SP	5	11 27	39 9	3 5	3 7	NO	
27	PTGV	Forest worker	M	28	SP	2	12 07	>37 4	7 5	2 4	P	
28	HLI	Seaside hotel manager	M	30	BU	9	12 30	40	2 5	1 4	P	
29	FJK	Taxi owner	M	37	BU	3	63 05 21	39	2 5	4 9	P	
30	HEK	Machinist	M	31	SP	6	05 30	39 5	6 2	6 6	P	
31	BRE	Farm owner	M	28	SP	1	07 01	> 40	12 5	10 2	P	
32	JAA	Housewife	F	38	SP	5	07 04	NI	+	1 8	P	
33	WIA	Lorry driver	M	31	SP	8	07 09	40 4	4 7	NPN	O	
34	KSB	Tractor driver	M	16	BU	6	08 02	39 5	4	2 4	P	

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
35	KHA	For st wo ker	M	6	BP	9	08 10	SE	10	1 1	NO	
36	DABA	Forest worker	M	39	EF	1	08 24	40 4	6 3	4 4	NO	
37	JFJ	Forest worker	M	23	BP	4	09 16	39 6	10	3 2	NO	
38	PSR	Farm owner/forest worker	M	62	BP	5	09 28	39	+++	3 2	NO	
39	BST	Laundress	F	50	BP	9	10 26	39	6 5	7 3	O	
40	KES	Forest worker	M	38	BP	4	11 06	—	3 3	1 6	P	Fever
41	BDT	Lorry driver	M	33	T	9	11 29	> 40	22	13 4	O/P	
42	BAA	Excavator	M	28	MD	7	12 01	40	+	3 8	NO	
43	MSV	Widow —	F	62	BP	1	12 03	—	1	MPH 145	MI	Lapp High fever
44	LX-O	Lorry driver	M	31	BP	9	12 21	39 6	5	7 2	O/P	
45	PBC	Telephone worker	M	53	T	9	64 03	11 39 6	4 5	5 9	MI	
46	LKH	Farm worker	M	59	BP	9	03 19	38	Med	8 1	MI	
47	DEV	Farm owner & wife	F	60	BP	10	04 22	38 5	3	2 8	NO	
48	AGFR	Student	M	16	BP	9	05 11	37 6	1	3 3	NO	
49	ELV	Student	M	21	T	6	07 20	40 1	10	4 5	O/P	
50	PPE	Farm owner	M	53	BP	9	10 13	> 40	2	6 0	MI	
51	LEA	Farm owner & wife	F	40	BP	5	12 28	40 6	MI	8 9	O	
52	HM E	Sawmill worker	M	16	BU	11	65 07 02	38 2	7 0	1 9	NO	
53	LIN	Industrial worker	M	25	BP	9	11 16	39 5	5	3 9	O/P	
54	LOG	Factory worker	M	35	BP	9	11 28	40	3	2 9	P	
55	LGM	Cook & assistant	P	55	BD	12	12 01	40	++	5 9	O	
56	DOT	Timber measurer	M	31	BU	11	12 17	40 3	0 3	6 2	P?	

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
15	HAGA	Forest worker	M	14	SP	5	08 02	39 4	+	0 8	NO	
16	AG	Forest worker	M	36	SP	2	08 25	41	+++	NPN 258	P	
17	GUI	Domestic help	F	22	SP	9	62 04 13	39 4	T	0 9	NI	
18	RK-A	Forestry foreman	M	37	BU	5	08 13	39 5	3	2 6	O/P	
19	S-AO	Canteen manageress	F	46	BU	9	08 25	40 4	+	1 1	NI	
20	NECA	Farm owner s/forest worker s wife	F	42	SP	9	09 06	39 9	4 5	6 1	O/P	
21	AKGO	Forest worker	M	28	SP	5	11 01	38 4	2	1 4	P	
22	NMK	Housewife	F	56	BU	9	11 02	39	4	1 2	NI	
23	DMA	Forest worker	M	41	SP	5	11 04	38 8	2 5	2 1	NO	
24	EBX	Cook s assistant	F	36	SP	9	11 09	>38 6	+	3 8	P	
25	HAE	Manager	M	48	SP	6	11 15	39	3 5	6 6	O/P	
26	ETH	Excavator	M	34	SP	5	11 27	39 9	3 5	3 7	NO	
27	PTGV	Forest worker	M	28	SP	2	12 07	>37 4	7 5	2 4	P	
28	HLI	Seaside hotel manager	M	30	BU	9	12 30	40	2 5	1 4	P	
29	PJK	Taxi owner	M	37	BU	3	63 05 21	39	2 5	4 9	P	
30	HEX	Machinist	M	31	SP	6	05 30	39 5	6 2	6 6	P	
31	BRE	Farm owner	M	28	BF	1	07 01	>40 12	5	10 2	P	
32	JAA	Housewife	F	38	SP	5	07 04	NI	+	1 8	P	
33	WIA	Lorry driver	M	31	SP	8	07 09	40 4	4 7	NPN 114	O	
34	KSB	Tractor driver	M	16	BU	6	08 02	39 5	4	2 4	P	

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
76	MDK	Fore te	M 50	SP		8	10 27	39	+++	5 4	O/P	
79	LKE	Tr sport lorry driver	M 40	T		9	11 01	39 5	2	5 9	P	Brother of no 75
80	LI	For st worker →	M 47	SP		1	11 01	39 1	5 0	4 0	P	
81	LSK	Carrier	M 52	SP		9	11 19	39	+++	1 5	MI	
82	LSM	Miner wife	F 46	BU		9	11 22	40	4	2 0	MO	High grade fever
83	EH	Forest worker	M 60	SP		6	11 26	→	1	9 0	P	
84	BMOI	Carpenter	M 35	BU		5	11 27	40	4 5	7 6	O/P	
85	LSA	Turner	M 31	BU		9	11 27	38 5	6	2 9	P	
86	BOL	Cableway mechanic	M 22	UV		5	12 09	38 8	5	5 8	P	
87	LJE	Mechanic	M 36	BU		4	12 18	40	4	2 2	P	High grade fever
88	EAM	Fore t/industrial worker	M 23	SP		5	12 19	→	+++	6 0	P	
89	EGI	Student	F 16	T		11	67 03 07	WT	++	BUM 23	MI	
90	LEA	Confectioner	M 59	T		9	03 11	> 38	+	4 8	O	
91	SHI	Sawmill/factory worker	M 41	SP		9	04 09	37 6	13 5	8 0	O	
92	PPLK	Machinist/tractor driver	M 23	SP		1	05 27	39 6	9 0	6 6	P	
93	FF	District medical officer	M 44	BU		2	06 30	39 5	+++	10 8	O/P	
94	BKOA	Electrician	M 41	T		11	07 26	40	+	1 2	MO	
95	LSPV	F rm owner	M 53	SP		9	07 30	38 8	23	9 0	O/P	
96	KLG	Political science student	M 24	T		11	08 17	40	5	1 8	O/P	

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
57	BLME	Housewife	F	49	SP	7	12 26	39	2	NPN 130	O/P	
58	EBEM	Nursing assistant	F	21	T	11	12 31	39 5	+++	2 1	NO	
59	HAG	Businessman	M	49	BU	5	66 01 18	40	+	1 1	O	
60	LBW	Miner	M	27	SP	5	04 03	40	7	4 0	P	
61	SRA	Farm owner	M	36	SP	9	06 01	39	+++	5 0	P	
62	ARA	Painter	M	29	BU	2	07 02	> 39	3 5	1 7	P	
63	BMJ	Tractor driver	M	27	SP	9	07 03	39 8	4 0	6 0	O/P	
64	OGSM	Bar assistant	P	34	SP	2	07 22	37 8	11	5 2	NI	
65	ASJA	Fitter	M	31	T	6	07 26	39	5	3 0	NI	
66	PEG	Foreman	M	33	BU	5	08 10	37 6	++	1 0	NO	
67	TH-G	Turner	M	18	BU	4	08 21	→	3 0	9 8	NO	Moderately elevated Conscript
68	EJE	Woodworker →	M	20	T	11	08 22	NE	2 8	2 7	P	
69	LBE	Policeman	M	39	T	11	09 06	39 8	+	2 0	P	
70	EH-E	Schoolboy	M	10	SP	1	09 16	39 5	3 2	5 1	NO	
71	JRA	Factory worker	M	33	SP	12	10 02	40 5	1 0	1 4	NO	
72	SEB	Motor mechanic	M	34	SP	7	10 06	39 6	+++	6 7	P	
73	LBK	Carpenter	M	19	BU	9	10 14	38 5	+++	1 8	NO	
74	MKG	Metalworker	M	31	SP	9	10 17	40	20	9 5	O/P	
75	LS	Garage attendant →	M	36	SP	1	10 21	38 2	12 5	10 0	O/P	Brother of no 80
76	NJE	Forest worker	M	28	BU	3	10 25	→	11 5	8 2	O/P	Fever
77	FUN	Lorry driver →	M	22	T	11	10 26	40 5	+	2 8	O	Conscript

()	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
78	MOX	Forester	M	50	SP	8	10 27	39	+++	5 4	O/P	
79	LXZ	Transport lorry driver	M	40	T	9	11 01	39 5	2	5 9	P	Brother of DO 75
80	LI	Forest work r →	M	47	SP	1	11 01	39 1	5 0	4 0	P	
81	LSK	Carrier	M	52	SP	9	11 19	39	+++	1 5	MI	
82	ABM	Miner's wife	F	46	BU	9	11 22	40	4	2 0	MO	High grade fever
83	EH	Forest worker	M	60	SP	6	11 26	→	1	9 0	P	
84	BMOI	Carpenter	M	35	BU	5	11 27	40	4 5	7 8	O/P	
85	LSA	Turner	M	31	BU	9	11 27	38 5	6	2 9	P	
86	BOL	Cableway mechanic	M	22	SP	5	12 09	38 8	5	5 8	P	
87	LJE	Mechanic	M	36	BU	4	12 18	40	4	2 2	P	High grade fever
88	EAM	Forest/industrial worker	M	23	EE	5	12 19	→	+++	6 0	P	
89	EGI	Stud nt	F	16	T	11	67 03 07	MI	++	BUM 23	MI	
90	LEA	Confectioner	M	59	T	9	03 11	> 38	+	4 8	O	
91	GHI	Sawmill/factory worker	M	41	SP	9	04 09	37 6	13 5	8 0	O	
92	PPLX	Machinist/tractor driver	M	23	BP	1	05 27	39 6	9 0	6 6	P	
93	FF	District medical officer	M	44	BO	2	06 30	39 5	+++	10 8	O/P	
94	BKOA	Electrician	M	41	T	11	07 26	40	+	1 2	MO	
95	LBPJ	Farm owner	M	53	SP	9	07 30	38 8	25	9 0	O/P	
96	KLG	Political science student	M	24	T	11	08 17	40	5	1 8	O/P	

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
97	SRE	Student	M	17	BU	4	12 28	40	17	1 5	NO	
98	SBA	Mining foreman	M	37	BU	9	58 10 18	> 39	4 5	5 7	O/P	
99	UKA	Drill worker	M	40	SP	5	11 13	40	++	2 9	O/P	
100	MEA	Shoemaker (retired)	M	53	BU	9	69 01 07	39	+	7 7	P	
101	JBH	Forest worker/farm owner	M	45	BU	9	01 14	40	3	3 2	P	
102	AKOA	Motor mechanic	M	19	BU	9	02 20	40 6	0 5	7 7	P	
103	DMY	Housewife	F	25	T	9	03 26	40 3	T	2 3	P	
104	HTG	Farm owner	M	27	SP	9	04 17	39 5	10	6 9	O/P	
105	LTC	Concrete worker	M	25	SP	5	07 10	39	0 5	6 5	NO	
106	EE	Nursing assistant	F	28	BU	5	08 27	40	12	12 6	O	
107	KASL	Hospital porter	M	30	T	9	09 24	40 1	16	3 4	O/P	
108	THBG	University student	M	23	T	11	10 06	39	++	11 0	O/P	
109	SLI	Mechanist	M	30	SP	5	10 06	40 6	4 5	8 2	O/P	
110	KIL	Farm owner & wife	F	36	SP	5	10 17	40 6	5 5	9 0	O/P	
111	EAA	Reindeer keeper	M	27	SP	1	10 20	38	2 6	1 8	F	
112	PTE	Forest worker	M	42	SP	1	10 23	NE	15 0	5 4	P	
113	LSU	Lorry driver	M	19	BU	2	10 29	39	+++	13 0	O/P	
114	SUC	Tractor driver	M	32	T	9	10 30	→	20 0	6 6	P	
115	TKR	Construction worker	M	28	SP	5	12 09	40	1 0	2 9	P	Fever
116	JSA	Chauffeur	M	23	SP	9	12 12	NE	5	5 3	O/P	
117	ABA	Secondary school student	M	20	BU	9	12 20	> 40	4	4 8	P	
118	HSC	Dustman	M	43	T	11	12 21	→	+++	3 2	P	Fever

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
119	HOQ-O Fore t worker		M	35	SP	7	12 24	41	+++	3 3	NO	
120	LK E Cl rk		M	40	BU	7	12 28	40	2 5	3 2	NO	
121	EJH Special subject teacher		M	45	BU	9	70 01 02	40	+	1 7	P	
122	HOQ-B Housewife		F	26	SP	9	01 03	40	+	4 0	NI	
123	WTH Truck driver		M	35	BU	12	02 14	39 3	+++	13 6	P	
124	HO-MK Forest worker s wife		F	29	SP	9	03 15	39 6	+	2 0	P	
125	EMK Forest worker s wife		F	55	SP	9	04 12	40	1	8 2	O/P	
126	HBK Retired		F	72	SP	9	05 21	39 8	3	14 0	O/P	
127	JYG Foreman		M	58	SP	3	06 11	40	+++	15 0	O/P	
128	LAR Tractor driver/farm owner		M	41	SP	9	06 19	40	10	5 7	P	Brother of no 131
129	ERG Forest worker →		M	23	SP	7	07 12	40	+	7 5	NO	
130	WKS Technical student		M	20	T	6	07 22	40 5	4 4	4 0	P	Brother of no 129
131	ERA Student →		M	19	SP	7	07 27	40 4	3 1	1 1	NO	
132	LK-O Forest worker		M	30	SP	7	07 30	40	3 0	7 3	P	
133	FREL Garage attendant		M	25	BD	3	08 08	NR?	+	12 2	P	
134	EM Supervisor s wife		F	42	T	9	08 07	40	+	4 9	O/P	
135	JG Sawmill worker		M	28	BU	4	08 17	> 40	2 5	40	P	
136	WS Hairdresser		M	54	T	11	08 17	41 1	+++	2 0	NO	
137	FTHD Electrician		M	32	SP	8	08 18	39 5	Neq	BDW 17	NO	
138	JSE Lineman		M	57	T	6	08 19	39 9	15 0	3 3	NO	

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
97	SRE	Student	M	17	BU	4	12 28	40	17	1 5	NO	
98	SSA	Mining foreman	M	37	BU	9	8 10 18	> 39	4 5	5 7	O/P	
99	OXA	Drill worker	M	40	SP	5	11 13	40	++	2 9	O/P	
100	MEA	Shoemaker (retired)	M	53	BU	9	69 01 07	39	+	7 7	P	
101	JSH	Forest worker/farm owner	M	45	BU	9	01 14	40	3	3 3	P	
102	AKOA	Motor mechanic	M	19	BU	9	02 20	40 6	0 5	7 7	P	
103	OMY	Housewife	F	25	T	9	03 26	40 3	T	2 3	P	
104	HTG	Farm owner	M	27	SP	9	04 17	39 5	10	6 9	O/P	
105	LTE	Concrete worker	M	25	SP	5	07 10	39	0 5	6 5	NO	
106	ES	Nursing assistant	F	28	BU	5	08 27	40	12	12 6	O	
107	KASL	Hospital porter	M	30	T	9	09 24	40 1	16	3 4	O/P	
108	PHBG	University student	M	23	T	11	10 06	39	++	11 0	O/P	
109	SLI	Machinist	M	30	SP	5	10 06	40 6	4 5	8 2	O/P	
110	KIL	Farm owner & wife	F	36	SP	5	10 17	40 6	5 5	9 0	O/P	
111	EAA	Reindeer keeper	M	27	SP	1	10 20	38	2 6	1 8	P	
112	PTE	Forest worker	M	42	SP	1	10 23	NE	15 0	5 4	P	
113	LSU	Lorry driver	M	19	BU	2	10 29	39	+++	13 0	O/P	
114	SHE	Tractor driver	M	32	T	9	10 30	→	20 0	6 6	P	
115	PKR	Construction worker	M	28	SP	5	12 09	40	1 0	2 9	P	
116	JSA	Chauffeur	M	23	BU	9	12 12	NE	5	5 3	O/P	
117	ABA	Secondary school student	M	20	BU	9	12 20	> 40	4	4 8	P	
118	HSE	Dustman	M	43	T	11	12 21	→	+++	3 2	P	Fever

()	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
												Conscrip
159	HMG	Cleaner →	M	19	T	11	12 01	38 6	++	3 8	MO	
160	WES	Foreman	M	35	BU	11	73 01 14	39 4	+++	4 7	P	
161	ETA	Welder	M	41	BP	7	01 16	> 39	+++	1 9	MO	
162	JAHM	Confectioner	M	39	T	9	01 22	39	0 8	12 3	P	
163	FMC	Housewife	F	33	T	6	01 23	> 40	1 5	12 0	P	
164	OAS	Farmer & wife	F	58	BU	7	01 31	38 5	0 9	7 3	P	
165	ABKS	Housewife	F	37	SP	4	02 05	39	+	6 5	P	
166	MSA	Farmer & wife	F	50	SP	5	02 10	NI	+++	12 3	O/P	
167	BSAR	Farm owner & wife	F	30	SP	9	03 05	39	1 9	4 8	P	
168	WMA	Horse help	F	19	BP	9	03 15	38 5	3	6 7	O/P	
169	FRJ	Cle k	M	25	T	11	03 20	39 5	++	3 5	P	
170	GBMA	Housewife	F	59	BP	1	03 26	40	3 3	5 2	O	
171	BR	Schoolboy	M	9	BP	9	04 11	39 8	6 5	2 2	MO	
172	LHM	Student	F	15	BP	9	04 22	40	0 7	9 4	O/P	
173	MAIE	Housewife	F	57	T	11	05 13	39	NEG	11 4	O/P	Son of no 176 and no 177
174	JSG	Student →	M	16	BP	7	05 20	40 4	++	5 4	P	
175	EB-GK	Colour sergeant	M	32	T	11	05 21	39	+++	5 1	P	Mother of no 174
176	JEF	Wife (of no 177) → Country division police commission- er's assistant/ farmer →	F	40	BP	7	05 31	39 7	++	6 1	P	Father of no 174, dialysed
177	JB		M	41	BP	7	06 06	39 8	+++	12 4	O/P	
178	APJ	Grinder	M	37	T	9	06 25	39 5	8	14 7	O/P	
179	PS-O	MI	M	26	T	11	06 27	39 9	+++	12 9	O/P	

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
139	LAA	Farm owner	M	33	SP	8	08 27	39 5	+	2 9	P	
140	HEA	Railway clerk	P	48	BU	9	08 30	39 5	5	6 2	P	
141	BEEL	Cook	P	40	T	9	09 02	39 5	7	3 2	O/P	
142	LKSS	Glassworker	M	30	SP	5	09 07	39 5	20	7 5	O/P	
143	OL-G	Student of philosophy	M	26	T	11	10 04	40 3	+	9 0	O/P	
144	MAB	Labourer	M	31	SP	3	10 11	> 39 15	0	7 3	O/P	
145	PKO	Labourer	M	22	BU	7	11 07	39 3	+++	6 9	O/P	
146	WJOG	Secondary school student	M	17	BU	7	11 22	41	3	3 2	NO	
147	BSE	Farm owner & wife	P	70	SP	5	11 25	41	+	3 4	O	
148	REE	Repairer	M	55	BU	11	71 02	28	39 9	+	7 2	P
149	BXE	Student	P	19	SP	2	04 23	NI	T	9 9	NO	
150	BEJ	Teacher (retired)	P	65	T	11	09 08	38 3	++	5 9	P	
151	LSS	School canteen manageress (retired)	P	62	SP	8	09 08	40	Neg?	11 3	P	
152	JFE	Building worker	M	60	T	11	10 15	40	++	BUW 23	NO	
153	NP-L	Welder	M	30	T	9	11 11	39 5	5	1 6	P	
154	VWJJ	Lorry driver	M	20	SP	9	12 23	38 8	15 5	2 2	NO	
155	ASSE	Caterpillar tractor driver	M	33	BU	2	12 26	39 9	+	9 6	O	
156	LEJ	School caretaker	P	50	T	11	72 08	14	37 7	+++	13 2	P
157	JAS	NI	M	18	BU	2	10 23	39 5	+++	5 2	P	
158	KEH	Shop assistant	P	50	BP	2	11 25	39 9	0 5	8 6	P	

Diaryed

()	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
			M 64	SP		4	10 19	39 6	4 3	9 0	O/P	Out-patient
202	BJE	Farm owner	M 23	BU		11	10 25	39 5	Meq	1 0	MI	Influenza
203	SOS	Service mechanic	M 58	SP		1	10 26	→	MI	3 5	P	
204	JWR	Farm owner	M 27	T		11	10 27	38 2	+++	1 6	P	Fever
205	SM H	Sorting clerk	M 33	SP		2	11 02	→	++	1 6	MI	
206	MRT	timber measurer	F 21	BU		7	11 03	40	+++	4 8	MO	High grade fever
207	EIO	Nursing assistant	F 48	BU		9	11 03	→	+	3 0	P	
208	GG	Housewife	F 22	SP		2	11 04	38 5	++	1 9	MO	
209	DJB	Tractor driver	M 48	SP		11	11 06	39 5	+	4 8	O/P	Crippled by cardiac val- vular disease
210	KOR	Housewife	F 51	T		11	11 10	40 4	+	2 3	P	
211	CCS	Plumber	M 51	T		11	11 11	41	++	1 1	O	
212	WST	Carpenter	M 32	SP		2	11 11	40 6	+++	2 3	P	Fever
213	ALS	Farmer	M 23	SP		2	11 12	→	21	12 1	P	
214	DOM	Welder	M 42	BU		9	11 14	39	+	3 4	P	Fever
215	LI	Housewife	F 37	BU		2	11 15	→	2 1	2 3	P	
216	LJQA	Factory worker	M 26	BU		9	11 15	SE	+	8 3	MI	
217	OSA	Housewife	F 40	SP		3	11 16	40	6 2	4 8	P	Fever
218	LS	Painter	M 22	T		9	11 17	→	3 5	1 6	MI	
219	CEG	MI	M 22	BU		3	11 17	39	++	3 7	P	High grade fever
220	DKL	Lineman	M 34	BU		2	11 20	→	MI	5 6	O	
221	NP	Forest worker	M 66	SP		6	11 26	→	9 5	8 5	O/P	Fever
222	LLG	Stockroom worker	M 35	BU		9	12 01	40	+	2 9	NO	
223	SRI	Railway man	M 23	BU		9	12 02	38 2	3 0	2 7	MI	
224	AB8	Forest worker	M 53	SP		6	12 02	40	+++	5 8	O/P	
225	ABC	Shopkeeper	M 41	BU		10						

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
180	BLAR	Self-employed	M	22	BU	5	07 03	40 11	+	3	5	P
181	KCA	Graduate in medicine	M	27	T	9	07 10	40	+	4	5	P
182	LSS	Engineer	M	28	T	11	07 21	39	+	5	6	P
183	WKG	Nursing assistant	F	24	T	11	07 27	39	TR	3	7	NO
184	BHH	Miner	M	29	BU	9	08 03	40	+	1	9	P
185	HA	Journalist	M	50	BU	7	08 17	41	++	5	3	P
186	VRV-A	Domestic help	P	23	BU	4	08 30	40 6	17 5	9	4	O/P
187	NY	Stockroom worker	M	21	BU	8	08 31	> 38	+	5	1	P
188	NBM	Housewife	P	37	T	9	09 03	39	+	6	9	P
189	LHI	Telecommunication technician	M	41	T	11	09 11	> 40	+++	11	4	P
190	LLH	Farmer	M	34	BU	9	09 12	→	+	3	3	P
191	JLMO	Carpenter	M	28	BU	5	09 12	40 9	+	10	7	P
192	HB	Housewife	P	41	BU	6	09 13	39 6	+++	16	0	O/P
193	JT	Student	M	24	SP	11	09 15	→	1 4	8	4	O/P
194	HKM	Student	F	19	T	6	09 20	40	+++	2	5	NI
195	HGA	Forest worker	M	47	SP	6	09 28	39	++	2	9	P
196	ELT	Baker	M	37	BU	4	10 01	40	0 5	4	4	O/P
197	IP	Tractor driver	M	42	SP	1	10 09	40	NI	4	6	NI
198	PJR	Farm owner	M	49	SP	9	10 12	→	15	4	1	NO
199	STB	Foreman	M	36	BU	9	10 12	→	4 5	8	4	P
200	HHL	Engineer	M	35	BU	11	10 15	39	TR	1	2	NO
201	RAG	Electrical engineer	M	58	BU	9	10 19	NI	+	6	1	NO

a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(k)	(l)	(m)	(n)
47	KLA	Joiner	M	22	SP	9	01 26	—	++	6 1	P	Fever
48	PNE	Upholsterer	M	44	SP	7	01 26	40 7	++	11 8	O/P	
49	EM	Petrol truck driver	M	28	T	11	01 31	40 3	+++	11 6	P	
50	LES	Metal worker	M	25	T	9	01 31	39 5	5 6	7 5	MI	Fever
51	HKFR	Factory worker	M	18	SP	9	02 02	—	NI	4 4	MI	
52	MRA	Taxidermist	M	35	SP	9	02 02	39 8	+	4 2	P	
53	LIV	Widow	F	86	BU	9	02 10	39	MI	2 7	NI	
54	AAV	Log-flo ter	M	65	SP	9	02 13	MI	+	8 5	NI	High grade fever
55	ALM	NI	P	29	SP	1	02 15	—	NI	8 8	O/P	Pensioner (epilepsy)
56	SEBS	Housewife	F	44	SP	9	02 15	40	6 4	6 3	O	Fever
57	HMOB	Tractor driver	M	30	SP	9	02 19	—	5 7	2 2	NI	
58	FU	Forest/farm worker	M	20	SP	1	03 06	39 5	4 0	15 2	O/P	
59	GEN	Schoolboy	M	10	BU	9	03 10	40	1 0	2 9	MO	Fever
60	LHS	Foreman	M	49	SE	9	03 10	—	2 5	5 0	NI	Influenza
61	LDA	Manager	M	42	T	9	03 11	—	1	9 0	P	
62	CEJ	Farm owner	M	55	SP	11	03 13	NE	++	6 5	NI	
63	IBBJ	Blast furnace worker	M	64	T	9	03 16	—	+	2 5	P	Fever
64	VEVI	Factory worker	M	31	BU	9	03 17	—	4 6	3 8	P	Fever
65	HGA	Housewife	F	58	SP	5	04 06	40	+	5 6	MO	
66	LKR	Student	M	27	T	9	04 11	—	7 5	3 7	O/P	Fever
67	HGE	Housewife	F	52	SP	9	04 26	39	23 5	9 2	NI	

(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(j)	(k)	(l)	(m)	(n)
226	HA	Motor winder	M	35	BU	9	12 03	—>	5 0	6 0	P	Fever
227	VEJO	Plastic worker	M	39	SP	9	12 04	—>	22 5	9 6	NI	High grade fever of no 231
228	LPMT	Schoolboy	M	8	SP	9	12 05	40 2	10	3 6	O	
229	WSG	Disabled pensioner —>	M	39	BU	5	12 07	—>	+	6 6	P	Mo Bechterew Shivers
230	HTG	NI	M	28	BU	3	12 12	—>	+	3 1	NI	Fever
231	VBU	Student	P	15	SP	9	12 12	39	+	3 8	P	Daughter of no 227
232	PK-I	Student	M	17	SP	2	12 12	NE	+++	3 0	NI	
233	HSI	NI	P	49	BU	6	12 15	—>	+++	8 2	P	High grade fever
234	HKGR	Welder	M	21	BU	9	12 17	39 7	12 0	6 7	O	
235	BR	Farm owner	M	48	SP	6	12 21	38	NI	2 0	NI	
236	HSI	Farmer	M	25	SP	10	12 31	39 5	+++	9 7	P	
237	LKGS	Metal worker	M	25	SP	9	74 01 13	—>	+	5 9	P	"Fever
238	FB-M	Shop assistant	P	30	SP	11	01 13	40	3 6	7 0	P	Fever Chron-ic myeloid leukemia (on prednisolone therapy)
239	PAK	Wood-carver —>	M	50	SP	9	01 16	—>	12	7 4	NI	High grade fever
240	LR	Boy	M	5	SP	9	01 18	SE	0 1	4 1	O	
241	BL-E	Office employee	M	29	SP	11	01 22	—>	+++	3 6	NO	
242	VGN	Accountant	M	29	BU	9	01 22	38 8	1 2	3 2	P	
243	OBW-O	Student	M	18	SP	9	01 22	NI	0 5	1 5	NO	
244	SRL	Welder	M	35	HP	9	01 23	—>	30	13 8	P	High grade fever
245	JRM	Chauffeur	M	30	BU	9	01 24	40	++	8 6	P	
246	LT	Technical student	M	26	SP	9	01 25	40	+++	1 6	NI	

Age- group	MALES				FEMALES				Bo h group			
	Age		Age		Age		Age		Age		Age	
	SP	BU	AC	SP	BU	AC	SP	BU	AC	SP	BU	AC
39	n P	520	9550	14757	4763	9738	14501	9970	19288	29256		
	P P	10 0	14 0	12 3	10 7	13 8	12 6	10 3	13 9	12 4		
	P P	28	32	60	7	3	10	35	35	26 2		
	P C	27 2	30 7	29 8	20 6	11 5	16 7	25 5	26 9	26 2		
	C/P	(0 373)+	(0 233)+	(0 282)+	(0 102)+	(0 021)	(0 048)+	(0 244)+	(0 126)+	(0 166)+		
49	n P	6914	9226	16140	6160	9173	15333	13074	18399	31473		
	P P	13 1	13 5	13 4	13 8	13 0	13 3	13 5	13 3	13 4		
	P P	18	13	31	7	8	15	25	21	46		
	P C	17 5	12 5	15 1	20 6	30 8	25 0	18 3	16 2	17 2		
	C/P	(0 181)	(0 098)	(0 133)+	(0 080)+	(0 061)+	(0 068)+	(0 133)+	(0 079)+	(0 101)+		
64	n P	10911	10781	21692	9202	10944	20146	20113	21725	41838		
	P P	21 0	15 8	18 0	20 6	15 5	17 5	20 7	15 7	17 8		
	P P	13	11	24	10	5	15	23	16	39		
	P C	12 6	10 6	11 6	29 4	19 2	25 0	16 8	12 3	14 6		
	C/P	(0 083)	(0 071)-	(0 077)-	(0 075)+	(0 032)+	(0 052)+	(0 079)-	(0 051)-	(0 065)-		
65	n P	7048	5972	13020	6105	7095	13200	13154	13066	26220		
	P P	13 5	6 8	10 8	13 7	10 1	12 5	13 6	9 4	11 2		
	P C	3	0	3	2	8	4	5	2	7		
	P C	2 9	0	1 4	5 8	7 7	6 6	3 6	1 5	6		
	C/P	(0 030)-	(0)	(0 016)-	(0 023)	(0 020)-	(0 021)-	(0 026)-	(0 011)-	(0 019)-		
tition P C	n P	52185	68123	120308	44658	70458	125116	96844	138581	235425		
	P P	100 0	100 0	100 0	100 0	100 0	100 0	100 0	100 0	100 0		
	P C	103	104	207	34	26	60	137	130	267		
	P C	100 0	100 0	100 0	100 0	100 0	100 0	100 0	100 0	100 0		
	C/P	(0 137)	(0 106)	(0 119)	(0 053)	(0 026)	(0 036)	(0 098)	(0 065)	(0 079)		

Mean population of AC county for the census years of 1960 1965 and 1970 (n P) living outside (SP) or inside (BU) built-up areas of the county (AC) and number of cases of EBN (n C) diagnosed December 1959 - April 1974; distribution on sex and age-groups

Ratio of (C/P) proportion of cases (p C) to the corresponding proportion of population (p P);

- the proportion of cases fell below or + above that of the population (Table IX page 30)

Within brackets is given the mean prevalence (number of cases per 1 000 inhabitants and year) of EBN for the period studied separately for each sub-group of the county population examined

Age-group	MALES			FEMALES			Both sexes		
	Area			Area			Area		
	SP			SP			SP		
	BU	AC		BU	AC		BU	AC	
- 9	n P	6558	11239	17797			12649	21976	34625
	p P	12 6	16 5	14 8			13 1	15 9	14 7
	n C	3	0	3			3	0	3
	p C	2 9	0	1 4			2 2	0	1 1
	C/P	(0 032)-	(0)	(0 012)-			(0 016)-	(0)	(0 006)-
- 19	n P	9269	10800	20069			17401	21770	39171
	p P	17 6	15 9	16 7			18 0	15 7	16 6
	n C	10	11	21			14	13	27
	p C	9 7	10 6	10 1			10 2	10 0	10 1
	C/P	(0 075)-	(0 071)-	(0 073)-			(0 056)-	(0 041)-	(0 048)-
- 29	n P	6279	10555	16834			10483	22357	32840
	p P	12 0	15 5	14 0			10 8	16 1	13 9
	n C	28	37	65			32	43	75
	p C	27 2	35 6	31 4			23 4	33 1	28 2
	C/P	(0 310)+	(0 243)+	(0 268)+			(0 212)+	(0 134)+	(0 159)+

Appendix Table C Mean prevalence (number of cases per 1 000 inhabitants and year) of EBN diagnosed among the inhabitants of the 12 municipal districts of AC county northern Sweden, December 1959 - April 1974 The population figure is the mean of that for the census years of 1960 1965 and 1970 respectively; cf also Fig 9 page 42

Municipal district	Sparsely populated area				Built-up areas				Entire district				Mean prevalence; ratio of district to the county mean	Land area; sq km	Mean number of inhabitants per sq km
	Mean pop- ulation	Nos of cases	Mean prevalence	Mean pop- ulation	Nos of cases	Mean prevalence	Mean pop- ulation	Nos of cases	Mean prevalence	Nos of cases	Mean prevalence	Mean pop- ulation			
1 Sorsele	3188	14	0 305	1822	1	0 038	5010	15	0 208			7494	0 67		
2 Storuman	15432	10	0 128	4196	8	0 132	9628	18	0 130			7485	1 3		
3 Vilhelmina	6074	3	0 034	3827	5	0 091	9901	8	0 056			8120	1 2		
4 Åsede	7210	4	0 039	3844	6	0 108	11054	10	0 063			7118	1 6		
5 Norsjö	7254	23	0 220	4397	8	0 126	11651	31	0 185			3384	3 4		
6 Lycksele	6811	7	0 071	8741	9	0 072	15552	16	0 071			5636	2 8		
7 Vindehn	5228	11	0 146	3432	7	0 142	8660	18	0 144			2727	3 2		
Inland region	41197	72	0 121	30259	44	0 101	71456	116	0 113			41964	1 7		
8 Värmdö	6893	5	0 050	5775	1	0 012	12668	6	0 033			1859	6 8		
9 Skellefteå	26711	52	0 135	45650	52	0 079	72361	104	0 100			6846	10 6		
10 Robertsfors	6045	2	0 023	2351	1	0 030	8396	3	0 025			1276	6 6		
11 Umeå	11263	5	0 031	50383	30	0 041	61646	35	0 039			2212	27 9		
12 Nordmaling	4892	1	0 014	3908	2	0 036	8800	3	0 024			1239	7 1		
Coastal region	55804	65	0 081	108067	86	0 055	163871	151	0 064			13432	12 2		
AC county	97001	137	0 098	138326	130	0 065	235327	267	0 079			53396	4 2		

FROM THE DEPARTMENT OF MEDICINE, KAROLINSKA INSTITUTET
AT SERAFIMERLASARETTET STOCKHOLM SWEDEN

Cholesterol and Bile Acid Metabolism in Normo - and Hyperlipoproteinaemia

by Bo Angelin

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- VII. AHLBERG J ANGELIN B BJÖRKHEM L and EINARSSON K.
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fasting man Submitted for publication.
- VIII. ANGELIN B and BJÖRKHEM L Postprandial serum bile acids in
healthy man - evidence for differences in absorptive pattern between
individual bile acids Gut in press (1977)

The papers will be referred to in the text by their Roman numerals as list
ed above

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ABBREVIATIONS

C	Cholic acid
CD	Chenodeoxycholic acid
D	Deoxycholic acid
GBD	Gallbladder disease
HDL	High density lipoprotein(s)
HLP	Hyperlipoproteinaemia
LCAT	Lecithin cholesterol acyl transferase
LDL	Low density lipoprotein(s)
VLDL	Very low density lipoprotein(s)

Cholesterol is of vital importance as a structural component of all cellular and intracellular membranes and serves as a precursor for steroid hormones and bile acids. The recognition of cholesterol as an important factor in the pathogenesis of clinically important conditions such as atherosclerosis and cholelithiasis has promoted a vast interest in the formation, transport, and degradation of cholesterol in man. As a contribution to our knowledge in this field, the aim of the investigations summarized in the present thesis was to study some aspects of cholesterol and bile acid metabolism in normo- and hypertipoproteinaemic man.

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VLDL	Very low density lipoprotein(s)

According to current concepts most if not all LDL is derived from VLDL breakdown via IDL (43, 60). LDL is the major vehicle for cholesterol in human plasma, and as evidenced from studies in cultured fibroblasts (23, 24) the uptake and degradation of LDL is critically dependent on a membrane surface receptor specific for these lipoproteins. In analogy with other metabolic receptors the "LDL receptor" itself appears to be subject to homeostatic regulation (21). The major site of LDL catabolism is not known, but there is some indication that the extrahepatic tissues may play an important role (103).

HDL (high density lipoproteins or lipoproteins) within the density range 1.063-1.21 are the smallest lipoproteins (90-120 Å). Protein (about 50%) and phospholipids (about 35%) are the major constituents, whereas cholesterol and triglycerides account for about 15 and 5% respectively. HDL are synthesized by the liver and the intestine (43, 60) contain apoproteins necessary for the lipoprotein lipase reactions and provide esterified cholesterol to VLDL and LDL by means of the LCAT (lecithin: cholesterol acyl transferase) reaction. HDL may also play an important role in the transport of cholesterol from peripheral tissues to the liver (80).

TRIGLYCERIDE METABOLISM

Dietary fat is hydrolyzed in the lumen of the small intestine and subsequently absorbed mainly as monoglycerides and fatty acids. Triglycerides are re-synthesized in the mucosal cells and secreted into the lymph vessels as chylomicrons and VLDL. The proportion between the amounts of fat transported in chylomicrons and in VLDL seems to be influenced by the amount of fat that has been absorbed (50). VLDL continue to be secreted from the intestine long after the disappearance of chylomicrons from the circulation.

In the liver triglycerides may be formed from de novo synthesized fatty acids and from free fatty acids liberated by lipolysis in adipose tissue. Under fasting conditions and during ordinary dietary conditions, plasma free fatty acids are virtually the sole precursors for hepatic VLDL triglycerides (55). After ingestion of carbohydrate-rich diets hepatic fatty acids appear to play a significant role in the formation of VLDL (79). The rate-limiting step(s) in triglyceride (and VLDL) synthesis are not known, and our insight

BACKGROUND

LIPOPROTEINS AND LIPOPROTEIN METABOLISM

Being insoluble in water lipids are transported in plasma bound to proteins. Free fatty acids are mainly complexed to albumin while triglycerides free and esterified cholesterol, and phospholipids are present in lipoproteins. In triglyceride-rich lipoproteins these complexes contain a core of the apolar lipids — triglycerides and cholesteryl esters — and a surface of the more polar lipids — free cholesterol and phospholipids — together with apoproteins (60-77).

Chylomicrons are the largest lipoproteins, with a diameter of 300-5000 Å and a density of less than 0.95 (g/ml). The protein content is only 0.5-2% while 80-95, 2-12 and 3-15% of the weight is made up of triglycerides, phospholipids, and cholesterol respectively (49, 60-77). Chylomicrons carry exogenous fat from the intestine to other tissues where the triglyceride core is hydrolyzed by the enzyme lipoprotein lipase. Due to rapid clearance from the circulation chylomicrons are not present in the plasma of healthy fasting man.

VLDL (very low density lipoproteins pre- β -lipoproteins) have a diameter of 280-700 Å and contain triglycerides (50-70%), cholesterol (10-25%), phospholipids (15-25%) and protein (10-13%) giving a density of 0.95-1.006 (49-60, 77). Most VLDL are synthesized in the liver but the intestine is also capable of VLDL formation (50). These lipoproteins transport endogenous triglycerides to other tissues especially adipose and muscular tissue where the removal mechanisms appear to be common for endogenous and exogenous triglycerides (25). During the breakdown of VLDL there is an exchange of apoproteins with other lipoproteins. The remnant particles IDL (intermediate density lipoproteins) deliver much of their cholesterol to the liver (45-60) and are subsequently degraded into LDL (43-60).

LDL (low density lipoproteins β -lipoproteins) have a density of 1.006-1.063 and a diameter of 210-250 Å. They are composed of 20-25% protein, 40-45% cholesterol, 20-25% phospholipids and only about 10% triglycerides (49-60).

of cholesterol is absorbed (18, 67-89) incorporated into chylomicrons (and VLDL) and finally delivered to the liver - a key organ in cholesterol metabolism. The hepatic cholesterol is used in the formation of plasma lipoproteins (about 8-11 mmol/day) and bile acids (about 1 mmol/day). Furthermore, about 2-5 mmol of cholesterol is excreted with the bile each day (34-104).

Although most tissues in man are capable of synthesizing cholesterol, hepatic and intestinal cholesterologenesis clearly predominate (34-104). The rate-limiting step in cholesterol biosynthesis has been located to the formation of mevalonate from HMG CoA (β -hydroxy β -methylglutaryl CoA) - a reaction which is catalyzed by the microsomal enzyme HMG CoA reductase (for a review see 93). This enzyme is subject to a sensitive feed-back control still incompletely known in its details. As chylomicrons seem to be more effective than VLDL in suppressing cholesterol synthesis in the rat liver, exogenous rather than endogenous cholesterol may be of major importance in this respect (111). Intestinal cholesterologenesis appears to be regulated primarily by bile acids excreted into the intestine (33), although intestinal cholesterol may have some effect too (96). Studies in the rat (53-97) and in man (4-30) indicate that bile acids also inhibit hepatic HMG CoA reductase by negative feed-back regulation. In several tissues cholesterologenesis appears to be depressed due to feed-back inhibition by LDL, mediated over the LDL-receptor mentioned above (10).

Only small amounts of cholesterol are excreted through the skin (15) and eliminated as steroid hormones in the urine (73) under steady state conditions. The total formation of cholesterol should thus equal the combined faecal excretion of bile acids and neutral steroids minus dietary cholesterol intake.

BILE ACID METABOLISM

Bile acids are formed in the liver from cholesterol. The first and rate-limiting step - the microsomal 7α -hydroxylation of cholesterol - is subject to feed-back inhibition by bile acids returning to the liver via the portal vein (for a review see 32). In man, the two primary bile acids formed are C (cholic acid) and CD (chenodeoxycholic acid) (Figure 2). Under normal conditions C represents about two thirds of the bile acids produced (37-109). Bile acids are secreted in the bile conjugated with glycine or taurine, stored in the gallbladder and subsequently released into the duodenum. After participation

into the mechanisms regulating triglyceride formation and its incorporation into VLDL is incomplete

Fatty acids liberated by hydrolysis of chylomicron and VLDL triglycerides by the action of lipoprotein lipase, are taken up and incorporated into storage triglycerides (92-110). The latter may be mobilized as free fatty acids in the lipolytic process, which is under intricate hormonal control (27). A triglyceride lipase of hepatic origin is present in post-heparin plasma; it has been suggested to be active in the degradation of IDL (60-65).

CHOLESTEROL METABOLISM

A highly simplified over-all schedule of cholesterol turnover in man is presented in Figure 1. Dietary cholesterol mixes in the intestinal lumen with cholesterol secreted in the bile and from mucosal cells (34). About 30 to 50%

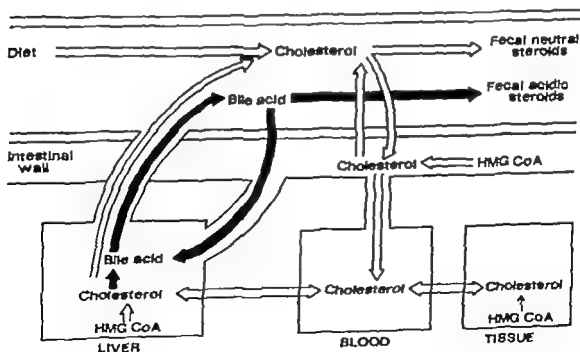


Figure 1. Cholesterol metabolism in man (see text)

of cholesterol is absorbed (18-67-89) incorporated into chylomicrons (and VLDL) and finally delivered to the liver - a key organ in cholesterol metabolism. The hepatic cholesterol is used in the formation of plasma lipoproteins (about 8-12 mmol/day) and bile acids (about 1 mmol/day). Furthermore about 2-5 mmol of cholesterol is excreted with the bile each day (34-104).

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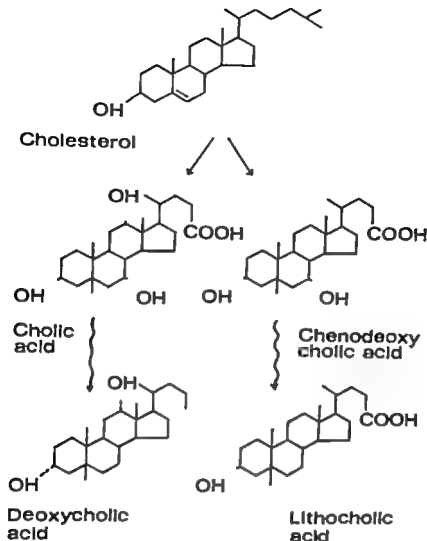


Figure 2 Formation of major bile acids in man (see text)

g in fat absorption in the upper small intestine bile acids are efficiently absorbed mainly by active transport in the distal ileum. Intestinal bacteria deconjugate and dehydroxylate the primary bile acids whereby D (deoxycholic acid) is formed from C and lithocholic acid from CD (Figure 2). The former is efficiently reabsorbed in the intestine while the latter is mainly lost in the faeces. Consequently the major bile acids present in human bile are C, CD and D normally in the proportions 1:1:0.9 (37). The ratio between C and CD pool sizes is often higher than the ratio between the concentration of the two acids in duodenal bile, a discrepancy suggesting that C and CD differ in rate of enterohepatic circulation.

Via the portal vein, the bile acids reach the hepatic sinusoids, where they are cleared by the liver and reexcreted in the bile. Virtually nothing is known about the portal venous concentration of bile acids in healthy man or about the extraction process its efficiency and possible differences between the individual bile acids. The concentration of bile acids in peripheral blood serum is very low (95) and little is known about its relation to the total bile acid content. The total bile acid pool undergoes continuous enterohepatic circulation, the daily excretion of bile acids in the bile amounting to about 50 mmol. Most of this material is reabsorbed and the amounts lost in the faeces of healthy subjects average 1 mmol/day (74)

HYPERLIPOPROTEINAEMIA

Serum lipoprotein levels may increase in patients with diseases such as diabetes mellitus hypothyroidism nephrosis biliary obstruction, and dysglobulinemia (47)

There are also primary forms of hyperlipoproteinaemia (HLP) i.e. disorders of familial or unknown origin. In 1967 Fredrickson et al (49) developed a system for the classification of primary HLP based on five phenotypes. Slightly modified (12) this system is now widely accepted, and its principal outlines are shown in Table 1

Table 1 Classification of hyperlipoproteinaemias (12)

Type	Lipoprotein elevated
I	Chylomicrons
IIa	LDL (β lipoprotein)
IIb	LDL + VLDL (β and pre- β lipoprotein)
III	IDL (floating β - or "broad β " lipoprotein)
IV	VLDL (pre- β lipoprotein)
V	chylomicrons + VLDL (pre β lipoprotein)

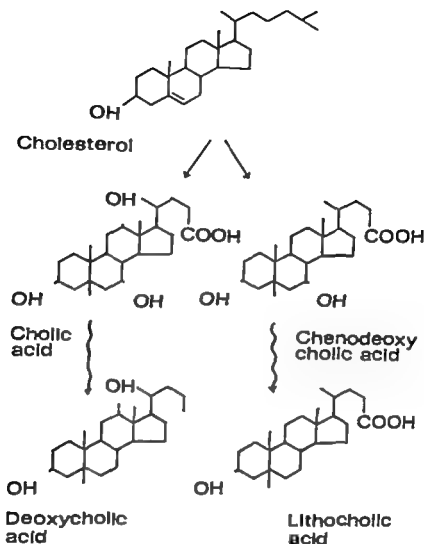


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HYPERLIPOPROTEINAEMIA

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An important discovery in recent years was the recognition that the identification of a phenotype does not necessarily define a genotype (51-78). Three different, apparently dominant genetic traits have been described: familial hypercholesterolaemia, familial hypertriglyceridaemia and familial combined hyperlipidaemia (51, 81). None of the traits was characterized by a specific lipoprotein pattern. The type II and IV lipoprotein phenotypes are the ones encountered most frequently (48-49), and the discussion below will be confined to these disorders.

Type II HLP This lipoprotein pattern may be seen in familial hypercholesterolaemia, familial combined hyperlipidaemia or polygenetic hypercholesterolaemia (78). In its classical form, familial hypercholesterolaemia is characterized by tendon xanthomata, corneal arcus and a high incidence of cardiovascular disease. According to *in vitro* studies the LDL elevation appears to be associated with defects in the LDL receptors. In cultured skin fibroblasts from patients with heterozygous familial hypercholesterolaemia, the content of LDL receptors is reduced by about 50% (20). Virtually no receptors may be found in the homozygous patient. The lipoprotein pattern in familial hypercholesterolaemia is generally that of type IIa, but occasional cases of type IIb are seen (22). In the other and more common forms of type II HLP, no LDL receptor deficiency has been described. Type IIa lipoprotein pattern is generally not associated with obesity, carbohydrate intolerance, or hyperuricaemia (22-48) which may be seen in some traits of HLP type IIb.

Type IV HLP The underlying metabolic defect(s) in type IV HLP have not been characterized and the clinical picture varies. Obesity and impaired glucose tolerance are encountered in about 50% of the patients. Hyperuricaemia is frequently observed and the incidences of GBD (41) and coronary heart disease are above normal (54).

Lipid and lipoprotein metabolism in HLP Studies of the metabolism of radio-labelled LDL in patients with heterozygous familial hypercholesterolaemia have revealed a decreased fractional turnover rate of the LDL particles (68-99). The formation of LDL appears to be normal.

The increased VLDL levels in type IV HLP may be due to overproduction of VLDL triglycerides (1-84, 87-90), decreased clearance (10, 35, 56, 88, 94) or both (62, 83). In agreement with the latter concept, kinetic studies with VLDL labelled in the apoprotein moiety (100) showed an increased formation of VLDL in six of eight subjects and a decreased clearance of the

lipoprotein in four VLDL-metabolism in type IIa HLP appears to be within normal limits (100)

Cholesterol synthesis has been reported to be normal in type II HLP (17, 52, 80, 106), while synthesis of bile acids was found to be subnormal normal (37, 74). The most apparent abnormality however is a reduced formation of C (37) resulting in a subnormal C-CD synthesis ratio

Type IV HLP is frequently but not invariably associated with an increased elimination of cholesterol as bile acids (37) mainly due to an elevated production of C. Similar findings with regard to faecal bile acid excretion have been reported (74). Apparently patients with type IV HLP are heterogeneous both with regard to triglyceride, cholesterol and bile acid metabolism.

CHOLESTEROL GALLSTONE DISEASE

Cholesterol secreted in the bile is rendered soluble by forming micelles with lecithin and bile acids (2, 91). Supersaturation of the bile with cholesterol may occur in response to factors influencing the secretion of bile acids, phospholipids and cholesterol disproportionately. According to current view supersaturation of the bile precedes and predisposes to formation of cholesterol gallstones in man (2, 91). The exact limits of cholesterol solubility - conveniently expressed as indices (2, 57, 59, 108) - are still under debate. An increased saturation of cholesterol in bile is often observed in obesity (8, 14) and during treatment with contraceptive steroids (13, 85) and clofibrate (86) i.e. conditions linked to an abnormally high incidence of gallstone disease (19, 29).

In a previous study (41) subjects with type IV HLP (in contrast to those with type IIa) appeared to carry an increased risk of gallstone formation. Patients with the type IIb pattern were not included in that study.

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MATERIAL AND METHODS

The subjects. The patients (I-VI) were those consecutively admitted because of HLP during the time of the study. The normolipidaemic control subjects (I-V, VI, VIII) were healthy volunteers, mainly members of the staff. The study reported in paper VII comprised patients admitted to the Department of Surgery for operation of uncomplicated gallstone disease. An informed consent was obtained from all subjects. Excluded were patients with HLP types III and V and those with evidence of intestinal, hepatic or renal disease, heart inc ompensation, hyper- and hypothyroidism and those addicted to alcohol or narcotics. In some studies (II-IV) patients with overweight ($> 120\%$ of ideal weight) were rejected.

Classification of HLP. WHO recommendations (12) were used for classification of HLP. Serum and β lipoprotein cholesterol, serum triglycerides and the lipoprotein pattern as evidenced by agarose electrophoresis were determined. The upper normal limit was set at 7.2 mmol/l for serum cholesterol (7.0 mmol/l in I), at 5.4 mmol/l for β lipoprotein cholesterol, and at 2.0 mmol/l for serum triglycerides.

Experimental procedure. The patients were hospitalized, and standardized dietary conditions were used (I-IV, VI). This diet was of natural type. The caloric intake was adjusted to keep the body weight constant. No antibiotics or other drugs known to affect lipid metabolism were given during the study or the preceding months. A detailed description of the experimental procedure is given in each paper.

Bile acid kinetics were determined after oral administration of [^{14}C] labeled C and CD. Duodenal bile was obtained through a thin polyvinyl tube in the fasting state at intervals of 1-3 days. The bile acid pool size, half life, and synthesis were determined as outlined by Lindstedt (71). [^3H] CD was originally used as tracer substance (I). It was later on replaced by [^{14}C] CD to avoid the slight error caused by non-specific losses of tritium in the body (38).

AIMS OF THE PRESENT STUDY

- 1 To study cholesterol metabolism in various types of hyperlipoproteinaemia by measuring the net steroid balance and the biliary lipid composition. (I, II)
- 2 To study the interrelationship between bile acid and endogenous plasma triglyceride metabolism in patients with different types of HLP (III, IV)
- 3 To determine the serum concentrations of individual bile acids in control subjects and patients with HLP (VI)
- 4 To characterize the enterohepatic circulation of individual bile acids (V VII, VIII)

GENERAL RESULTS

CHOLESTEROL METABOLISM IN HLP (I, II)

Net steroid 'balance' Altogether 69 subjects were studied with regard to the faecal excretion of neutral steroids (cholesterol, coprostanol and coprostanone) and the formation of C and CD. The intake of cholesterol was about 0.25 mmol/day. In the control subjects the faecal excretion of cholesterol and its neutral metabolites averaged 1.07 ± 0.13 mmol/day. Similar values were observed in HLP type IIa and type IIb, whereas the patients with type IV lipoprotein pattern tended to have a higher excretion of neutral steroids (mean 1.48 ± 0.17 mmol/day).

In the control subjects as well as in the patients with type IIa and type IIb HLP the formation of bile acids (C + CD) averaged about 1 mmol/day. Type IV HLP was associated in general with an increased bile acid synthesis 2.31 ± 0.22 mmol/day. As seen in Figure 3 the total elimination of cholesterol was similar (about 2 mmol/day) in the controls and the patients with type IIa and IIb HLP. Approximately equal amounts of cholesterol appeared to be eliminated as bile acids and neutral steroids. As described previously (37) the formation of C represented about 85% of the total bile acid synthesis in the controls but only 47% in subjects with type IIa and type IIb HLP. A clearly different pattern was seen in the patients with HLP type IV. Mainly due to the increased formation of bile acids (Figure 3) the cholesterol elimination was almost twice as high as that encountered in the control subjects. C accounted for 70% of total bile acids produced (cf. 37).

Neglecting urinary and skin excretion of steroids the net 'balance' was calculated as bile acid synthesis + faecal neutral steroid excretion - dietary intake. The balance averaged 1.83 ± 0.22 mmol/day in the controls 1.60 ± 0.15 and 1.81 ± 0.19 mmol/day in HLP type IIa and type IIb, respectively. The net 'balance' in HLP type IV was significantly higher 3.53 ± 0.23 mmol/day than that of the controls ($p < 0.001$). However the former group was heterogeneous and in about 50% of the patients the net steroid balance was within the normal range. Net steroid 'balance' did not correlate with

Neutral faecal steroids Pooled faecal samples collected during 7 to 10 days were used, in order to minimize the effects of irregular faecal flow. The neutral steroids (cholesterol, coprostanol and coprostanone) were determined by gas-liquid chromatography after extraction and isolation by thin layer chromatography (44)

Endogenous plasma triglyceride kinetics were determined as described by Farquhar et al (46) and later on by Nikkilä and Kekki (83, 82, 83). [$2-^3\text{H}$] glycerol was injected intravenously after an overnight fast. Venous blood samples were collected repeatedly and analyzed for plasma radioactivity and triglyceride concentration. Analysis of the radioactivity decay curve was performed with a digital computer. Only single exponential curves were accepted. Altogether about 10% of curves were rejected for this reason or because of unstable triglyceride concentrations.

Serum bile acids An assay based on combined gas chromatography - mass spectrometry was developed (VI)

Details of the analytical procedures employed are given in the individual papers

Statistical procedure Data are presented as mean \pm SEM. The χ^2 test, Student's t test and paired t test and the Wilcoxon rank sum test have been used (102). Linear regressions have been calculated with the method of least squares and their significances tested by estimating the correlation coefficient r (102).

molar percentage of cholesterol, bile acids and phospholipids in duodenal bile was 7.2 ± 0.2 , 71.2 ± 0.7 and $21.6 \pm 0.6\%$ respectively. As compared to HLP type IIa, HLP type IIb and type IV were associated with an increased molar percentage of cholesterol $10.2 \pm 0.8\%$ and $9.9 \pm 0.6\%$ respectively. The lithogenic indices according to Admirand and Small (2) and Hegardt and Dam (57) and Holzbach (58) were also significantly higher in both type IIb and type IV than in type IIa HLP ($P < 0.001$).

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On page 20, the first four 1 composition and the occurrence

The occurrence of GBD was de patients consecutively admitted frequency of GBD was 8, 18 and females with type IIa, IIb and Altogether 138 patients were 40.

The formation of plasma triglycerides was significantly higher in type IIa HLP. The corresponding values obtained in type IIb ($20.7 \pm 1.9 \mu\text{mol kg}^{-1} \text{ h}^{-1}$) and type IV HLP ($22.1 \pm 1.4 \mu\text{mol kg}^{-1} \text{ h}^{-1}$) were significantly higher. In general, males tended to produce more triglycerides

sex, glucose intolerance, body weight serum cholesterol or triglyceride concentration.

Biliary lipid composition and the occurrence of GBD in HLP (II)

The occurrence of GBD was determined in 210 hyperlipoproteinaemic patients. Frequency of GBD was 8.18 and 42% in males and 22.48 and 72% in females patients were 40-59 years old. The incidence of GBD in this group was compared with the findings in three age-matched necropsy series from Malmö General hospital (72/107/112) and was significantly higher in the male ($P < 0.001$) and the female ($P < 0.025$) patients with type IV HLP. A tendency to an increased frequency of GBD although not statistically significant was also seen in females with type IIb HLP.

Altogether 38 non-obese hyperlipoproteinaemic patients without GBD were studied with regard to bile lipid composition. In type IIa HLP the mean

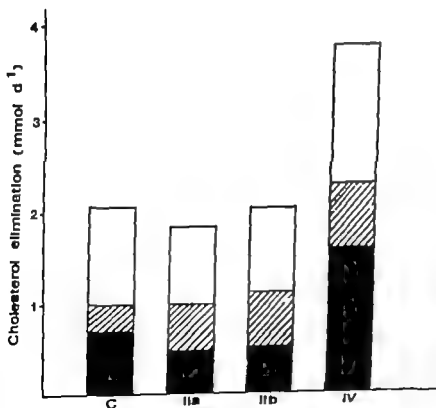


Figure 3 Elimination of cholesterol (mmol d^{-1}) in control subjects (C) and in patients with hyperlipoproteinaemia

■ cholic acid ▨ chenodeoxycholic acid □ faecal neutral steroids

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Excretion: Patients with type IIa or IIb HLP eliminated normal amounts of cholesterol as bile acids and faecal neutral steroids. More than half of patients with type IV HLP displayed a net steroid balance above the normal limit. The average ratio between the amount of C and CD synthesized was subnormal in HLP type IIa and type IIb but supranormal in HLP type IV.

Type IV is to a high extent associated with an increased saturation of cholesterol in the bile. This finding may have some bearing to the increased prevalence of GBD in patients with this disorder.

BILE ACID AND PLASMA TRIGLYCERIDE KINETICS IN HLP TYPE IIb AND IV (III, IV)

Under basal conditions (III). Bile acid and plasma triglyceride kinetics were determined in 4 patients. In agreement with previous studies (37, cf I) the formation of bile acids was lower in type IIa (0.72 ± 0.07 mmol/day) and type IIb (0.83 ± 0.12 mmol/day) than in type IV HLP (1.43 ± 0.16 mmol/day). This difference was mainly due to an enhanced production of C which averaged 0.95 ± 0.11 mmol/day in type IV and 0.42 ± 0.04 mmol/day in type IIa. As evident from figure 4 the differences between the types of HLP concerning bile acid production were still present when expressed as $\mu\text{mol kg}^{-1}$.

The formation of plasma triglycerides averaged $10.5 \pm 0.7 \mu\text{mol kg}^{-1} \text{ h}^{-1}$ in type IIa HLP. The corresponding values obtained in type IIb ($20.7 \pm 1.9 \mu\text{mol kg}^{-1} \text{ h}^{-1}$) and type IV HLP ($22.1 \pm 1.4 \mu\text{mol kg}^{-1} \text{ h}^{-1}$) were significantly higher. In general males tended to produce more triglycerides.

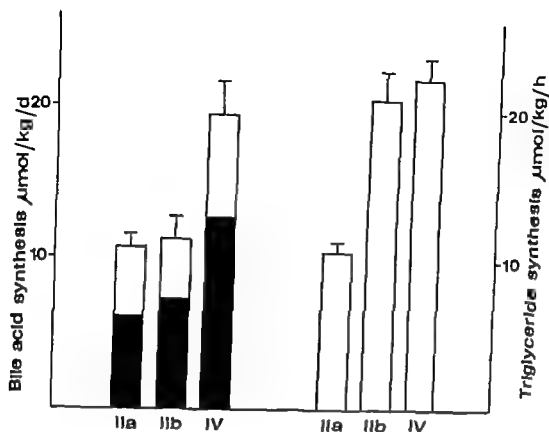


Figure 4 Synthesis of bile acids (□ cholic acid, ■ chenodeoxycholic acid $\mu\text{mol/kg/d}$) and triglycerides ($\mu\text{mol/kg/h}$) in patients with hyperlipoproteinaemia type IIa, IIb and IV

than females. The mean fractional turnover rate of triglycerides was $0.188 \pm 0.008 \text{ h}^{-1}$ in type IIa and $0.177 \pm 0.011 \text{ h}^{-1}$ in type IIb. Significantly lower values $0.147 \pm 0.011 \text{ h}^{-1}$ were encountered in HLP type IV.

A positive correlation was obtained between the formation of plasma triglycerides and that of C in HLP type IIa ($r = +0.69$, $P < 0.001$) and type IV ($r = +0.70$, $P < 0.001$). A similar relationship was seen for (C + CD) with $r = +0.59$ ($P < 0.01$) in type IIa and $r = +0.69$ ($P < 0.001$) in type IV HLP. The production of CD and triglyceride correlated in HLP type IV ($r = +0.67$, $P < 0.01$). No such relationship was found in the patients with the type IIa pattern.

Treatment with cholestyramine and CD (IV) The triglyceride formation was reinvestigated in 26 hyperlipidaemic subjects. For 2-4 months before the second study 14 of the patients had been treated with cholestyramine (12 g/day). The other 12 patients had been administered CD (1 g mmol = 750 mg/day).

Previous data have demonstrated that cholestyramine treatment results in a stimulation (40) and CD therapy in a depression (61) of the bile acid production. During administration of cholestyramine, the formation of plasma triglycerides increased in all patients with HLP type IIa, the means before and during therapy being 9.7 ± 1.2 and $12.8 \pm 1.5 \mu\text{mol kg}^{-1} \text{h}^{-1}$ respectively. Cholestyramine treatment also stimulated the fractional turnover rate, which increased from 0.176 ± 0.014 to $0.230 \pm 0.017 \text{ h}^{-1}$. In the five patients with type IV HLP studied, cholestyramine therapy had no consistent effects on plasma triglyceride kinetics.

During feeding with CD, the synthetic rate of plasma triglycerides decreased in subjects both with HLP type IIa (from 13.1 ± 1.2 to $7.9 \pm 0.5 \mu\text{mol kg}^{-1} \text{h}^{-1}$) and type IV (from 23.6 ± 3.7 to $15.5 \pm 1.8 \mu\text{mol kg}^{-1} \text{h}^{-1}$). In the whole series of patients the fractional turnover of plasma triglycerides decreased from 0.169 ± 0.013 to $0.133 \pm 0.013 \text{ h}^{-1}$. A 15% reduction of plasma triglyceride levels was seen.

Conclusions. Under basal conditions in patients with HLP type IIa or IV the rate of degradation of cholesterol to bile acids appears to reflect the rate of plasma triglyceride formation. Upon stimulation of bile acid production by a bile acid sequestering agent the production of plasma triglycerides becomes enhanced in HLP type IIa. Treatment with CD that primarily may inhibit bile acid biosynthesis results in a decreased production of plasma triglycerides both in HLP type IIa and IV.

INDIVIDUAL SERUM BILE ACIDS (VI)

Method. Mass fragmentography (combined gas chromatography - mass spectrometry) was used for the determination of C, CD and D in serum. [2, 2, 3, 4, 4 - H_2] C was used as internal standard for C and [11, 11, 12 - H_3] D as internal standard for both CD and D. The bile acids were hydrolyzed, extracted, methylated and converted to trimethylsilyl ethers. In the mass fragmentographic assay C was quantitated from the ratio between the tracing at m/e 368 ($M - 3 \times 90$ of derivative of unlabelled C) and that at m/e 363 ($M - 3 \times 90$ of derivative of deuterium labelled C). Accordingly D was quantitated from the ratio between the tracing at m/e 370 ($M - 2 \times 90$ of deriva

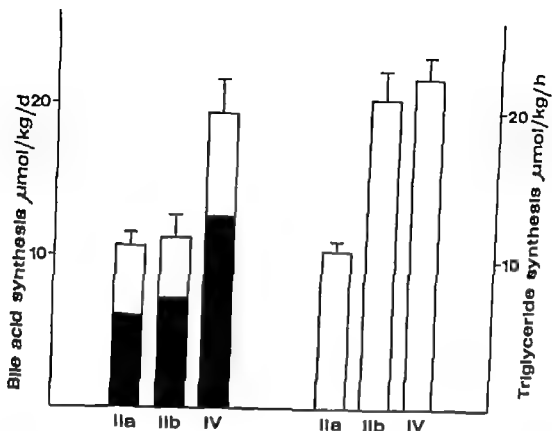


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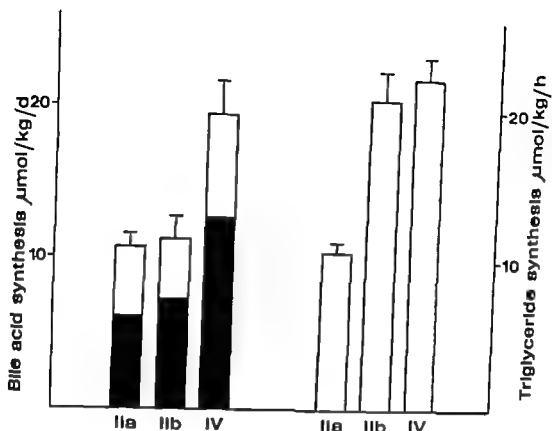


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Conclusions. A method was developed for assay of individual bile acids in serum using mass fragmentography. The serum concentration of C is decreased in type IIa and IIb HLP but within normal limits in HLP type IV. Serum levels of C reflect C pool size in controls and type IIa HLP.

ENTEROHEPATIC CIRCULATION OF INDIVIDUAL BILE ACIDS (V-VII, VIII)

Absorption of bile acids in the proximal small intestine (V) In 22 subjects (5 controls, 8 with type IIa and 9 with type IV HLP) samples of intestinal content were drawn through a double-lumen tube, with the upper hole located at the ligament of Treitz (80 cm from the teeth) and the lower one 100 cm further down. The relative bile acid composition (C:CD:D, %) in the samples drawn from the 80 cm level averaged 37:31:32%. The corresponding figures in samples from the 180 cm level were 43:27:30%. Thus, the ratio between C and CD was considerably higher 1.73 ± 0.16 in the samples from the 180 cm level compared to those drawn at the 80 cm level 1.34 ± 0.08 . Disregarding the uptake of C, the mean absorption of C and CD along the segment studied was estimated to 28 and 23% respectively.

Portal venous and systemic serum bile acids (VII) Portal venous serum concentration of C averaged $6.14 \pm 1.20 \mu\text{mol/l}$ in 10 subjects undergoing elective cholecystectomy. The CD concentration was higher ($8.40 \pm 1.84 \mu\text{mol/l}$) while D showed intermediate levels (Figure 5). A highly significant difference was encountered between the bile acid concentration of portal and peripheral venous serum where C, CD and D levels averaged 0.49 ± 0.16 , 1.55 ± 0.32 and $1.44 \pm 0.59 \mu\text{mol/l}$ respectively (Figure 5). No differences were found between arterial and venous blood serum. Neglecting the probable difference between peripheral and hepatic venous bile acid concentrations, the fractional hepatic uptake was estimated to be $92 \pm 2\%$ for C and $78 \pm 3\%$ for CD.

The relative composition of bile acids in hepatic bile (C:CD:D, %) was 35 ± 5 , 31 ± 2 , 35 ± 6 while the contribution of CD was significantly greater in portal venous serum where the proportions were 30 ± 3 , 39 ± 2 , 31 ± 5 .

Postprandial serum bile acids (VIII) The response in individual serum bile acid concentrations to a standardized meal was studied in five healthy subjects. Fasting serum concentrations of C, CD and D averaged 0.45 ± 0.06

tive of unlabelled D) and that at m/e 373 ($M - 2 \times 90$ of deuterium-labelled D) and CD from the ratio between the corresponding tracing at m/e 370 ($M - 2 \times 90$ of derivative of unlabelled CD) and that at m/e 373 ($M - 2 \times 90$ of derivative of deuterium-labelled D)

The standard curves obtained showed that these ratios were linear with the respective bile acid concentration up to about $20 \mu\text{mol/l}$. The accuracy of the method was tested by adding various amounts of bile acids to serum samples with known concentrations of bile acids. The error in the determinations was less than 10% for C and CD and slightly greater in the determination of high amounts of D. The relative standard deviation (calculated from duplicate determinations of different serum extracts) was 3.4 and 7% for C, CD and D respectively.

Serum bile acid concentrations In the control subjects ($n = 21$) the concentration of C averaged $0.47 \pm 0.06 \mu\text{mol/l}$ while those of CD ($1.45 \pm 0.15 \mu\text{mol/l}$) and of D ($1.12 \pm 0.11 \mu\text{mol/l}$) were significantly higher. In samples drawn at an interval of 15-30 min in the fasting state the coefficient of variation in the determination of C, CD and D was 4.3 and 3% respectively. The corresponding coefficients in samples drawn in the fasting state one week - three months apart were 13.21 and 35%.

In type IIa HLP ($n = 32$) the serum levels of C ($0.30 \pm 0.03 \mu\text{mol/l}$) and CD ($1.06 \pm 0.11 \mu\text{mol/l}$) were significantly lower than those encountered in the controls. A similar pattern was seen in patients with type IIb HLP ($n = 10$) whereas those with HLP type IV ($n = 32$) did not differ from the normal lipidaemic controls. Bile acid concentration did not correlate to sex, body weight or serum lipid levels. The serum concentration of D in patients with GBD was higher than that in the total series of patients.

Relation to bile acid kinetics Altogether 42 subjects were included in this study. The serum level of C correlated positively to the C pool size in the controls ($r = +0.93$, $P < 0.025$) and in the patients with type IIa HLP ($r = +0.69$, $P < 0.005$). No significant correlation was seen in type IV HLP although the patients with this disorder appeared to form two subgroups. In about half of the patients the serum concentration seemed to correlate with the pool size. The serum concentration of CD did not show any correlation to the CD pool size in any of the subgroups. Bile acid formation did not relate to serum levels.

GENERAL DISCUSSION

The importance of the enterohepatic circulation of bile acids in the regulation of lipid metabolism is well documented (for review 32-58). The biosynthesis of the primary bile acids is controlled by the amounts of bile acids returning to the liver in the portal vein (feed back inhibition). The fractional turnover rate of C is in general higher than that of CD (37). The current investigation indicates that these two primary bile acids also differ with regard to the site of their intestinal resorption. This difference, which results in a slower enterohepatic circulation of the trihydroxy cholanoic acid C than of the dihydroxy cholanoic acid CD, may have some implications physiological as well as methodological. CD in contrast to C appears to suppress the hepatic secretion of cholesterol (3-69-70). Moreover in numerous studies dealing with bile acid kinetics the ratio between the concentrations of individual bile acids in the bile has been used as an index of the relation between the sizes of their pools. Such indices and data recorded for the pool size of one bile acid have been used in the calculation of the total amount of bile acids in the enterohepatic circuit. On the basis of the present findings it appears beyond doubt that a proper determination of the total bile acid pool size requires determinations of the pool sizes of the individual bile acids.

The bile acids reaching the liver in the portal vein are cleared from the circulation very rapidly. C more efficiently than CD and D. The concentration of bile acids in peripheral blood serum is thus very low and accounts for only a minute part of the total pool. The easy accessibility of serum bile acids has made them a subject of much interest, particularly since radioimmunoassay techniques were introduced (101). The level of an individual bile acid in serum is mainly determined by the momentary balance between input from intestinal absorption and the output due to hepatic clearance (cf. 58). Elevated concentrations of bile acids in serum in hepatocellular disease has been recognized since long (26-98) but the factors determining the levels in fasting healthy man have not been subject to much study.

In the present investigation, the concentration of CD in peripheral venous serum was about three times that of C, a consequence both of the more rapid enterohepatic circulation and of the less efficient clearance. The concentration of C in serum was related to the pool size of C both in controls and in the patients with type IIa HLP. Thus the lower serum level of C

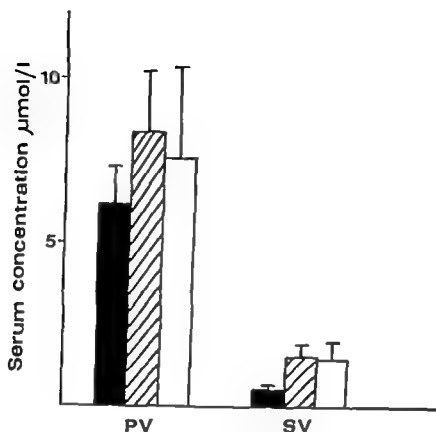


Figure 5: Serum concentration of bile acids

(■ cholic acid, ▨ chenodeoxycholic acid, □ deoxycholic acid μmol/l) in portal (PV) and systemic vein (SV)

1.06 ± 0.11 and 1.22 ± 0.21 μmol/l respectively. A significant rise in serum CD and D was seen already 30 min after the meal with a CD level twice that seen in the basal state. C showed a significant increase in serum concentration after 60 min. The peak levels were seen after 90 min for all bile acids with the concentration of C averaging 1.01 ± 0.07 that of CD 3.04 ± 0.23 and that of D 2.24 ± 0.26 μmol/l. After 150 min, the C concentration had returned to basal level whereas the CD concentration was still significantly higher than basal.

Conclusions The serum level of C is normally lower than that of CD. This finding may have a dual explanation. Firstly, CD circulates through the enterohepatic system at a higher rate than C. Secondly, the hepatic clearance of C exceeds that of CD.

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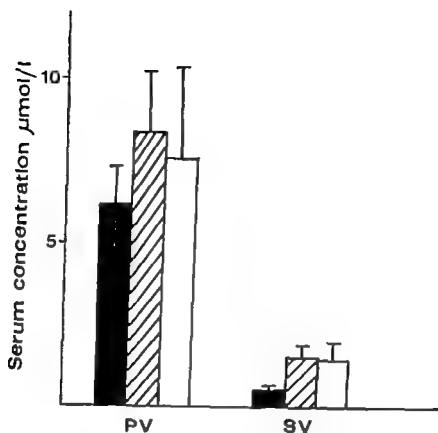


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cholestyramine increases bile acid synthesis while excretion as faecal neutral steroids is unaffected (74). Experiments in the rat have demonstrated that de novo formed cholesterol is preferred for the synthesis of bile acids (11-31) especially for that of C (76). Preliminary data from our laboratory suggest that this also applies to man (8). If so the synthesis of C would appear to be an 'index' of the hepatic cholesterogenesis. VLDL cholesterol and bile acids (especially C) may thus in part originate from the same pool of newly-synthesized cholesterol. This hypothesis is further supported by the finding that stimulation as well as suppression of the bile acid formation is associated with reciprocal changes in the plasma triglyceride turnover.

The finding of an elevated cholesterol saturation in the bile and an increased occurrence of GBD in the patients with type IV HLP is of major interest. This indicates that GBD may be the manifestation of a more generalized metabolic disorder. The parallel changes in VLDL concentration (75) and biliary cholesterol saturation (9) in patients with type IV HLP during treatment with CD attract further attention to the possible role of a deranged lipoprotein metabolism as a factor in the evolution of supersaturated bile.

encountered in the latter group reflects the reduced size of the C pool demonstrated in previous work (37). In HLP type IV however large pool sizes of C were not associated with elevated serum levels of this bile acid. Disregarding the possibility of an increased clearance, the present evidence does suggest a reduced efficacy for the uptake of C in some patients with type IV HLP. In accordance with this concept a decreased capacity to retain orally administered C in these patients has been observed (39).

The elimination of cholesterol as bile acids is within the normal range in HLP type IIa and type IIb but above normal in about 50% of the patients with the type IV lipoprotein pattern (37). The various types of HLP also differ with regard to the formation of individual bile acids: the production of C being subnormal in HLP type IIa and type IIb but abnormally high in HLP type IV. The present study demonstrates that the amount of cholesterol excreted as bile acids roughly reflects the rate of cholesterol biosynthesis. In agreement with the former observation the activity of the rate-determining step in cholesterol biosynthesis, HMG CoA reductase, has recently been demonstrated to be about twice as high in the liver from patients with type IV HLP as compared to those with normal lipids (5).

As a general finding HLP type IV turns out to be heterogeneous with regard to defects in the metabolism of cholesterol and bile acids. Similarly a heterogeneous pattern has been recorded in studies of lipoprotein (apo B) and triglyceride kinetics. Under basal conditions the formation of bile acids correlates with the production of plasma triglycerides *i.e.* VLDL. Further support for the concept of a linkage between the formation of VLDL triglycerides and that of cholesterol and bile acids is gained from studies on the effects of treatment with hypolipidaemic drugs or diets. The reduced triglyceride synthesis induced by weight reduction (85) or by the administration of clofibrate (83) or nicotinic acid (84) to patients with the type IV lipoprotein pattern is accompanied by a decreased formation of bile acids (36, 37, 42). The increased triglyceride formation during feeding with a high carbohydrate diet (79) appears to be linked to an increased degradation of cholesterol to bile acids (7). There is also some evidence that the turnover of plasma triglycerides correlates with that of plasma cholesteryl esters and presumably to hepatic cholesterol biosynthesis (86).

It has been suggested that at least two hepatic cholesterol pools are present: one 'anabolic' for lipoprotein synthesis and one 'catabolic' derived from lipoprotein breakdown and used for elimination as bile acids and neutral steroids (104, 105). However, several lines of evidence suggest that the hepatic pool of cholesterol available for bile acid synthesis is not identical with that of cholesterol excreted into the bile. Accordingly the administration of

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respectively. Lower values were seen in patients with type IIa and type IIb HLP.

In controls and in patients with type IIa HLP the serum concentration of C correlated to C pool size. No such relationship was seen in HLP type IV.

4. The ratio between C and CD concentrations were determined in intestinal aspirates from different levels of the proximal small intestine. There was an increased C/CD ratio in samples from distal parts consistent with CD absorption of about 30% along the segment studied.

Portal venous serum was analyzed for bile acids and concentrations six to ten times those of peripheral serum were observed. The fractional hepatic uptake was estimated to be circa 90% for C and about 80% for CD and D.

The serum bile acid levels were followed during a standardized meal. CD (and D) showed an earlier and more prolonged rise in serum concentration compared with C. The maximum level was reached after about 60 min for all three acids.

GENERAL SUMMARY

- 1 Bile acid kinetics (isotope dilution) faecal neutral steroid elimination (gas chromatography) and cholesterol intake were determined under basal conditions in 69 normo- and hyperlipoproteinaemic subjects. The net steroid 'balance' calculated as bile acid synthesis + faecal neutral steroid excretion - dietary intake averaged 1.8 mmol/day in controls and in patients with type IIa and IIb HLP. Type IV HLP was frequently associated with an increased 'balance' mean 3.5 mmol/day mainly due to an increased production of bile acids.

In altogether 210 patients with HLP the incidence of GBD was determined and compared to the findings in three age-matched necropsy series. An increased occurrence of GBD was seen in patients with type IV lipoprotein pattern. The biliary lipid composition was analyzed in patients with HLP. Both type IIb and type IV HLP displayed an increased saturation of cholesterol in the bile compared to type IIa HLP.

- 2 Bile acid kinetics (isotope dilution) and plasma endogenous triglyceride turnover (^3H glycerol technique) were determined in patients with type IIa, IIb and IV HLP under basal conditions. In agreement with previous studies the formation of C was higher in type IV than in type IIa or IIb HLP. A correlation between the formation of bile acids (especially that of C) and the synthesis of plasma triglycerides was present in type IIa and type IV HLP.

Triglyceride kinetics were determined before and during induction of an increased bile acid formation by cholestyramine treatment. In patients with type IIa HLP plasma triglyceride formation and fractional turnover rate increased while no consistent changes were seen in type IV HLP.

A similar analysis of triglyceride kinetics before and during suppression of bile acid synthesis by CD feeding showed a decreased formation of triglycerides both in type IIa and type IV HLP.

- 3 A mass fragmentographic technique for determination of serum bile acids was developed and evaluated. The fasting concentrations in serum of C, CD and D averaged 0.47 ± 0.06 , 1.45 ± 0.16 and 1.12 ± 0.11 $\mu\text{mol/l}$.

respectively. Lower values were seen in patients with type IIa and type IIb HLP.

In controls and in patients with type IIa HLP the serum concentration of C correlated to C pool size. No such relationship was seen in HLP type IV.

- 4 The ratio between C and CD concentrations were determined in intestinal aspirates from different levels of the proximal small intestine. There was an increased C/CD ratio in samples from distal parts consistent with CD absorption of about 30% along the segment studied.

Portal venous serum was analyzed for bile acids and concentrations six to ten times those of peripheral serum were observed. The fractional hepatic uptake was estimated to be circa 90% for C and about 80% for CD and D.

The serum bile acid levels were followed during a standardized meal. CD (and D) showed an earlier and more prolonged rise in serum concentration compared with C. The maximum level was reached after about 60 min for all three acids.

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SEVENTY-YEAR-OLD PEOPLE IN GOTHENBURG
A POPULATION STUDY IN AN INDUSTRIALIZED SWEDISH CITY
II GENERAL PRESENTATION OF SOCIAL AND MEDICAL CONDITIONS

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ABSTRACT

The prospective longitudinal population study of elderly individuals in Gothenburg started in 1971/72 with an investigation of a representative sample (about 3/10) of the 70 year-olds. This wide population study concerns the nature and manifestations of normal ageing processes, the incidence and prevalence of disease at higher ages and the occurrence and the nature of infirmity in old age. This paper is a general description of some of the social and medical observations obtained during the first study of the probands and concerns migration in the population, housing, dwelling conditions, mobility

and transportations social contacts need of help results of the general othological ophthalmological psychiatric psychological and odontological examinations as well as of certain laboratory analyses The results showed that underdiagnosis was common in this age group i e that previously unknown disease often was present The results also indicate that overdiagnosis is rather common due to the fact that the border zone between normal ageing and disease is only a very vague presentiment in the higher age groups More advanced handicaps were rather uncommon and only about 3 per cent of the 70-year-olds suffered from handicaps or diseases to such an extent that care within institutions was necessary The 70-year-olds rather commonly are living under social conditions that from a medical point of view might be subjected to critizism It should however also be emphasized that a majority of the 70-year-olds in Gothenburg were living a very comfortable life in rather good physical and mental condition

INTRODUCTION

There are obvious and urgent needs for more knowledge of the nature and manifestations of normal ageing processes at higher ages as well as of the incidence and prevalence of disease at higher ages and of the occurrence and nature of infirmity in old age From a medical and sociological point of view an improvement of that knowledge is necessary for an adequate planning of service care and the prevention of disease and handicaps among the elderly

A broader knowledge in medical gerontology and geriatrics in humans necessitates epidemiological studies. The prospective longitudinal population study of elderly individuals in Gothenburg, the second largest city of Sweden, was started in 1971/72 by an investigation of a representative sample (3/10) of the 70-year-olds (29). The first follow up started in September 1976 at the age of 75 and simultaneously another study of possible cohort-effects in a 5-year-period has been started by an investigation of another sample of individuals who were 70 years old in 1976/77.

A great many specialists in different fields of gerontology and geriatrics (29) are taking part in this study. More detailed presentations obtained in different research fields will be published separately. The aim of this report is to introduce in a more general way the social and medical observations obtained during the first study of the probands who in 1971/72 were 70 years of age.

METHODS

The design and procedure of the study, analysis of observer variation and a comparison between responders (85 per cent) and non-responders (15 per cent) have been reported previously (29). This population study comprised a homecall part regarding basic personal data: dwelling conditions, economy, social and physical communications, previous migration, educational level, previous and any present professions, need of care, consumption of health care and drugs. An examination at the hospital comprised broad medical examinations.

and transportations social contacts need of help results of the general othological ophthalmological psychiatric psychological and odontological examinations as well as of certain laboratory analyses The results showed that underdiagnosis was common in this age group i e that previously unknown disease often was present The results also indicate that overdiagnosis is rather common due to the fact that the border zone between normal ageing and disease is only a very vague presentiment in the higher age groups More advanced handicaps were rather uncommon and only about 3 per cent of the 70-year-olds suffered from handicaps or diseases to such an extent that care within institutions was necessary The 70-year-olds rather commonly are living under social conditions that from a medical point of view might be subjected to criticism It should however also be emphasized that a majority of the 70-year-olds in Gothenburg were living a very comfortable life in rather good physical and mental condition

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common (43 per cent) size of flat 26 per cent had one room and kitchen and the rest 3 or more rooms. About one third had been living in the same house for 20 years or more but nearly as many (28 per cent) had moved into their present home during the last 5 years. Eightyfour per cent of both male and female probands considered their housing standard good 11 per cent acceptable and 5 per cent bad.

The general hygienic standards of the flats were usually good e.g. 93 per cent had a WC 90 per cent warm water and 93 per cent bathrooms thereof 83 per cent in their own apartments. It should be mentioned that more probands considered the indoor temperature during winter-time too high (12 per cent) than too low (6 per cent) an observation of special interest nowadays when energy saving programmes are actual. More women (25 per cent) than men (18 per cent) complained of draughts in their apartments.

Seventythree per cent lived in buildings without lifts and only 6 per cent of the homes were situated on the ground floor. Twelve per cent had a WC on another floor than that where the bedroom and kitchen were situated.

During the visit by the nurse the probands were asked to sit down in their usual armchairs and the lighting was measured at the level where they usually hold their books or papers when reading. The intensity of illumination was measured upon a special surface with a central indicator for light direction and shadow length. In 72 per cent of the cases the illumination was less than 300 lux in 13 per cent less than 100 lux - in 18 per cent 300-500 lux and in only 10-15 per cent of the cases lighting was considered to be adequate at the age of 70. The illumination was thus definitely not adjusted to the needs of the elderly. Furthermore approximately 70 per cent had an inconvenient direction of the artificial light with risk of dazzle and inferior

dental examination psychological examination dietary
interview anthropometric measurements and a body
composition examination

RESULTS

Gothenburg has apparently had a relatively stable population. Fiftyone per cent of the male 70-year-olds and 45 per cent of the females were born there and 11 per cent of the males and 16 per cent of the females born elsewhere had lived there for more than 50 years. Only 5 per cent of the males and 7 per cent of the females had lived there less than 20 years. Only 3 per cent of the 70-year-olds were born abroad and almost all of these had lived in Sweden for a sufficiently long time to obtain Swedish nationality. The majority (55 per cent) of the 70-year-old population in Gothenburg were women. 45 per cent of the female probands were living alone compared to only 19 per cent of the males. Among males only 12 per cent were widowers. Among females 42 per cent were widows. Only 7 per cent of the 70-year-olds lived in extended families 1 or 2 together with 3 or more persons.

Housing

Almost 4 out of 5 probands were living in flats in general apartment houses and only about 10 per cent were still living in their own family houses. Five per cent had moved to a housing unit for pensioners or a nursing home. The living room and a kitchen was the most

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Only 4 per cent lacked a telephone and only 14 per cent radio and/or TV

As their main transportation method within Gothenburg three out of four probands used the public transportation facilities and only 10 per cent had their own cars. Bus or tram stops were available within 400 metres for 80 per cent of the probands. About 10 per cent of the probands declared that walking was their main method of transportation. Sixtyone per cent declared that they were not used to making long (≥ 2 weeks) journeys in spite of the costs of public transportation being subsidized for pensioners in Sweden. Ten per cent go abroad occasionally.

Twentyseven per cent owned a summer cottage and another 16 per cent used childrens friends or rented cottages during summer months.

Social contacts

Two out of three probands had children alive and the vast majority (83 per cent) of these children were living in or close to Gothenburg. Eighty per cent had met their children within the last week. Fifteen per cent complained of meeting their children too seldom. Only 3 women thought that they met their children too often. Obviously children and grandchildren constitute the most common means of contact and only 17 per cent of those with children declared that their most common personal contact was with friends or relatives other than children. One per cent had no regular personal contact at all. More than one third of the probands felt that personal contact had become less common after retirement although available time had increased and one fifth declared that they had a larger circle of

friends now after retirement than before

Many foreigners feel that the average Swede is silent reserved and difficult to come into contact with. Among the 70-year-olds 70 per cent declared that they never paid a visit to a neighbour and 10 per cent that they did not even talk to neighbours when they met them in the apartment house. Ninety per cent declared however that they found their frequency of contacts with neighbours satisfactory.

On the other hand 38 per cent of the females and 21 per cent of the males complained of a feeling of loneliness sometimes (29 per cent and 17 per cent) or often (9 per cent and 4 per cent) and 20 per cent stated that they felt themselves lonely more often at the age of 70 than they had experienced at the age of 60. Further processing of the material has shown that the majority of probands with a subjective feeling of loneliness really were lacking personal contact and that it was not unusual especially among females that one or more days passed when they did not meet or talk to anyone at all. The group of 70-year olds who complained of loneliness will be studied separately with an aim to analyze from social, psychological and medical points of view the reasons for this loneliness and possibilities of improving the living conditions of these people.

Need of help

A thorough study was performed on the presence of handicaps as judged from the capability of the proband to exist in his/her own environment. Only 3 per cent were living in hospitals, nursing homes or homes for the elderly. According to our evaluation the 97 per cent living at home were able to cope but 2 per cent needed regular help with personal hygiene, dressing etc.

Eighteen per cent needed domiciliary help. Half of those needing help at home had got such help from the community services and 30 per cent had arranged help privately. Only 9 per cent of those living at home but needing help got such a help from their children. As mentioned earlier, out of those with children 80 per cent of the children were living in Gothenburg or its surroundings and might thus from a geographical point of view have been able to assist their parents. It was apparently more common that parents helped children; 20 per cent helped their children often, 20 per cent sometimes and 9 per cent seldom.

Ninetyfive per cent of the probands living at home were able to take a bath without any help. 99 per cent managed to go to the toilet and 70 per cent were doing their laundry themselves. Eleven per cent of the females and 5 per cent of the males had difficulties in rising from bed and 14 per cent and 6 per cent respectively rising from a chair without arms and 8 per cent and 5 per cent respectively of walking easily. Thirteen out of 973 investigated probands used wheelchairs and only 2 individuals of those living in their own homes were bedridden. Six per cent of the females and 4 per cent of the males had extra support from the community for the taxi transports or special handicap transports.

Reading habits were dominated by daily newspapers. 94 per cent had at least one newspaper and periodicals were procured by 67 per cent at least once a week. Fortyeight per cent had no other hobby than reading. Thirtyeight per cent declared that they never read a book (including the Bible). Only 8 per cent went to church every week and 44 per cent never went to church at all. About 50 per cent were members of a society and 60 per cent of those probands went to society meetings at least once a month.

Medical examination

The general medical examinations were performed by Dr Sture Roupé and Dr Bertil Steen and the roentgenological studies by Dr Erich Deichgräber

Eighty-eight per cent of males and 85 per cent of females had been admitted to hospital sometime during their life. Examples of the anamnestic life time incidences of diseases are given in Table I. Thirty-three per cent of the male and 36 per cent of the female probands declared that they did not feel fit. It should be noted that a great many of those who reported they felt fit later on responded in the affirmative to certain symptoms on disease when confronted with them. Eighteen per cent of the males and 25 per cent of the females considered themselves to suffer from a general tiredness and had done so for at least one year.

At the medical examination a great number of observations on symptoms of definable diseases were noted. More detailed descriptions of these findings will be given when the follow up of 1976/77 has been completed. The present report has therefore been limited to examples on preliminary evaluations of the prevalence of certain diseases in this age group (Table I). Congestive heart failure was defined as 1) roentgenological evidence of increased heart volume plus two of the symptoms oedema, dyspnoea and cyanosis or 2) all the three symptoms: oedema, dyspnoea and cyanosis or 3) roentgenological evidence of pulmonary congestion or 4) roentgenological evidence of increased heart volume in the absence of systemic hypertension (23).

Early systolic murmur of an ejection character with a maximum over aorta but without lowering of the second heart sound and supposed to indicate a lowered elasticity of aorta was heard in 25 per cent of the males and 37 per cent of the females.

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	<u>Males</u>	<u>Females</u>
Constipation laxative consumption	13	21
Gall stone anamnestic	14	29
Inguinal hernia	29	0
Mammary carcinoma	-	3
Urinary incontinence		
at cough and/or laughter	6	44
at night	2	1
Catheter or uridome	1	1
Bacteriuria	2	9
Suspect carcinoma of prostata	8	-
Definitive enlargement of prostata	46	-
Urgent miction	25	24
Diabetes		
anamnestic	6	6
oral treatment	4	5
insulin treatment	1	1
Joint diseases		
back pains every week	16	28
arthralgia every week	16	27
arthralgia knee joints	5	8
Rheumatoid arthritis	3	6
Heberden s nodules	3	10
Abnormally functioning		
fingers	8	9
wrists	2	4
elbows	3	4
shoulders	4	5
hips	7	9
knees	5	10
ankles	3	4

Table I Prevalence in per cent of certain symptoms or diseases Anamnestic = life time incidence

	<u>Males</u>	<u>Females</u>
Eczema	20	18
Psoriasis	3	1
Petechia	3	6
Vascular spiders	26	4
<i>Chronic bronchitis (WHO definition)</i>	18	9
Pulmonary carcinoma (X-ray)	2	0
Pulmonary metastases (X-ray)	1	0
Pulmonary tuberculoses active (X-ray)	0	0
Pulmonary tuberculoses inactive (X-ray)	74	80
Myocardial infarction (Minnesota code)	6	3
Probable myocardial ischemia (Minnesota code)	13	10
Angina pectoris (according to Rose)	13	10
Atrial fibrillation	4	2
Ventricular extrasystole	5	5
Atrio-ventricular blockade	5	2
Congestive heart failure	20-25	20-25
Intermittent claudication	■	7
Hypertension		
anamnestic	23	48
treated	12	30
Cerebrovascular disease		
anamnestic	2	1
hemipareses	1	1
Headache daily	2	6
Headache at least once a week	8	15

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Arcus corneae was observed in 71 per cent of males and 59 per cent of females and both arcus corneae and xanthelasmata in 7 per cent and 6 per cent respectively. Cutaneous and/or tendon xanthoma were not observed in any of the patients.

Fifty per cent of the males and 12 per cent of the females were smoking daily. 76 per cent of the male and 64 per cent of the female smokers smoked 5 or more cigarettes per day. 9 and 15 per cent more than 15 cigarettes per day. Thirtythree per cent and 6 per cent had been smokers earlier, but had now given it up. In 18 per cent of the males and in 9 per cent of the females anamnestic evidences of chronic bronchitis according to the WHO-definition were present and 20 per cent and 8 per cent respectively showed objective symptoms at the clinical examination. At the roentgenological examination of the lungs pulmonary emphysema was diagnosed in 9 per cent and 1 per cent active inflammatory disease in 2 per cent and 1 per cent respectively while changes considered to be due to earlier active but at the time of the present investigation inactive inflammatory disease were observed in 14 per cent and 12 per cent. Roentgenological changes indicating earlier tuberculosis were observed in 74 per cent of the males and 80 per cent of the females. Primary pulmonary malignant tumors were discovered in 2 males and pulmonary metastases in one man.

Histograms for systolic and diastolic (phase 4) blood pressure for males and females are shown in Figures 1 and 2.

Six per cent of the males and 3 per cent of the females had according to the Minnesota code (32) electrocardiographic evidences (Q of types I or II) of previous myocardial infarction. These figures would have increased to 15 per cent and 11 per cent respectively if the occurrence of a pathological Q of type III had been +

as evidence for myocardial infarction Thirteen per cent of the males and 10 per cent of the females suffered from anginal pain as defined by Rose (31) while 8 per cent and 4 per cent suffered from electrocardiographic changes of probable ischemia Four per cent and 2 per cent had atrial fibrillation 5 and 5 per cent ventricular extrasystole and 5 and 2 per cent respectively atro-ventricular blocks

As far as symptoms of cerebro-vascular diseases were concerned 1.7 per cent of the males and 1.3 per cent of the females reported that they had been or were suffering from such diseases while 1.2 per cent and 3.0 per cent had hemiparesis at the examination

Intermittent claudication was on the other hand registered in the same frequency (6 per cent) among males and females

Fourteen per cent of the males and 29 per cent of the females had had or were suffering from symptoms of gall stone 25 per cent and 10 per cent of peptic ulcer and 4 per cent of males and 0.2 per cent of the females reported that they had hernia while such hernia was diagnosed in no less than 29 per cent of the males (0 per cent of the females) at the clinical examination

Twentyfive per cent of the males and 24 per cent of the females complained of precipitate micturition Six per cent of the males and 44 per cent of the females complained of urinary incontinence with a cough or a laugh etc and 2 per cent and 1 per cent of incontinence in the night Two and 9 per cent had significant bacteriuria Twentyfive per cent of the males had apparent dysuria 46 per cent apparent enlargement of the prostata and 8 per cent suspected and later on by biopsy verified carcinoma of the prostata

Twentyone per cent of males and 34 per cent of females

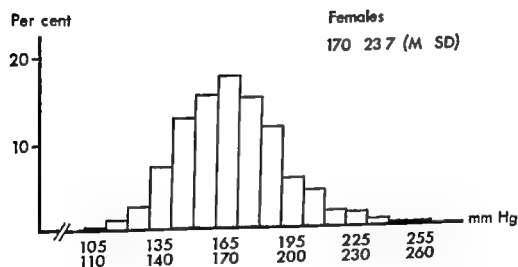
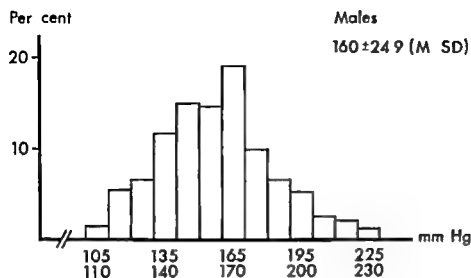


Figure 1 Systolic blood pressure

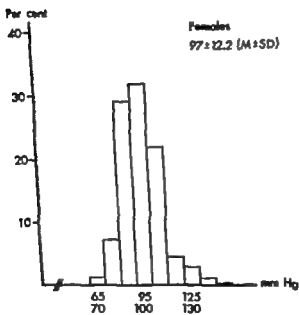
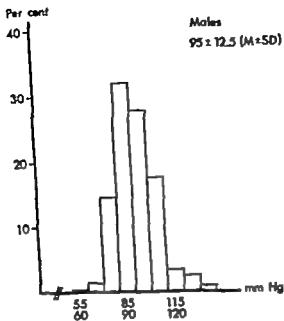


Figure 2 Diastolic (phase 4) blood pressure

reported back pains thereof 16 per cent and 28 per cent every week Sixteen per cent and 27 per cent complained of pains in peripheral joints 5 per cent and 8 per cent in the knee-joints

The 3 cases of mammary carcinoma were small and found in soft tissue X-ray examination of the breasts By the palpatory method suspect tumors were observed in 3 other women but further investigations by X-ray and biopsy showed that these abnormalities were benign Malignant tumors or highly suspected malignant tumors were observed on an average of 7 per cent of the probands

With mobility tests performed according to the method described by Gillner et al (15) 94 per cent of the males and 91 per cent of the females showed no limitation of mobility (0 points) 3 and 5 per cent showed some limitation (1 point) The highest number of scores observed was 32 points 1 = advanced handicaps

According to the nursing load point scale by Rultén et al (20) 94 per cent of the males and 91 per cent of the females showed 0 points 3 per cent and 5 per cent 1 point and the maximum points registered were 30 and 29 respectively

Dr Margareta Bjurö was responsible for the othological examination In conventional hearing tests 60 per cent of males and 86 per cent of females were able to hear whispering at a distance of 5 metres 29 per cent and 10 per cent ordinary conversation at this distance but 10 per cent and 3 per cent only at 1 metre distance Among the males 13 per cent and among the females 04 per cent could thus not hear ordinary conversation at a distance of 1 metre Tone and speech audiometry showed a lowering of the hearing ability of 50 decibel for speech in 8 per cent of the males and 1 per cent of the females 1 = on average in 5 per cent of the

As examples of retrospective longitudinal data obtained will be mentioned that almost all of the males (99.8 per cent) and half of the women (46 per cent) had had gainful employment and that the nature of their work and also the environment where they had been working was registered as carefully as possible between ages 20-40, 40-60 and after 60 as well as reasons for early retirement pensions. The results of global evaluation of health, cognitive function and social condition in relation to previous or present occupational work will be published separately.

The ophthalmological investigations performed by Dr Margareta Munkby showed e.g. chronic conjunctivitis in 11 per cent, grey cataract with a visual acuity of < 0.5 on one eye in 4 per cent and on both eyes in 1 per cent, glaucoma in 1 per cent and advanced macular degeneration in 2 per cent. Reduced visual acuity with maximum correction of glasses to 50-70 per cent of normal capacity was found in 9 per cent, to 20-40 per cent in 2 per cent and to 5-10 per cent in 1 per cent. The examinations showed that 95 per cent were able to read the telephone book in Gothenburg and 97 per cent the standardized text on bottles and other packagings of drugs in Sweden.

The psychiatric examination performed by Dr Göran Persson showed 1) Psychotic state (i.e. advance mental disturbances that made it impossible for the patient to take care of himself and/or the patient no longer realized himself that he was sick); schizophrenic - paranoid psychosis 1 per cent, organic lesions (senile dementia, arteriosclerotic dementia, traumatic or alcoholic lesion) 1.5 per cent. 2) Neurotic state (i.e. mental disturbances of slighter grade than the above mentioned); Organic lesions (see above) 3 per cent, anxiety - depression - compulsory neurosis 9 per cent.

both organic and affective illness 1.8 per cent 3) Furthermore certain individuals had mental symptoms apparently due to special personality traits The psychiatric observations will be published in detail separately

The odontological study was performed by Dr Tor Österberg and will also be published in detail separately These reports will be given in odontological journals which are not usually available to others than odontologists and the results will therefore be described somewhat more detailed below Thirtytwo per cent of the 70-year-olds who underwent the odontological examination stated that their own teeth or jaws caused inconvenience and 45 per cent considered dental care necessary Almost one third of both the males and females chewed their food with difficulty Every fourth woman experienced a dryness of the mouth In this matter there was a significant sex difference ($p < 0.01$)

Almost 60 per cent of the 70-year-olds had one or more subjective sign of maxillary joint pains difficulties when opening the mouth and when swallowing and chewing pains all of which pointed to the fact that functional disturbances of the chewing apparatus were common

Out of the 385 examined 70-year-olds lack of own teeth on the upper and lower jaw was observed in 69 per cent and 52 per cent respectively 51 per cent were lacking teeth in the upper as well as in the lower jaws

Thirtyfour per cent of the males and 26 per cent of the females had their own teeth in both upper and lower jaw Nineteen per cent of both the males and the females had their own teeth in only one jaw Mean value of own teeth in the probands with such teeth was 13 More than half of the 70-year-olds had less than 15 own teeth Almost 2/3 of these teeth had had preserving

treatment. The females had on average more repaired teeth than had the males. Mean value for number of teeth with caries was 2.8.

Eight per cent among those without teeth had been rehabilitated with upper jaw denture as early as before the age of 20. 60 per cent before the age of 50. Loss of own teeth of the lower jaw came later than of the upper jaw. Women lost their own teeth earlier in life than men. Teeth in the upper as well as the lower jaw. Almost half of those with complete dentures had had the same dentures for more than 10 years and one fourth had had the same dentures for more than 20 years.

As few as 14 per cent of those with complete dentures had well functioning dentures. Fifty-six per cent had dentures with an unacceptable function of the upper as well as of the lower jaw and an incorrectly balanced bite. Fifty-four per cent of the females and 80 per cent of the males had an unsatisfactory hygiene of the dentures. A significant difference between the sexes showed up ($p < 0.01$).

It is a wellknown fact that there is a relation between bad hygiene of denture, bad fitting dentures and changes of the mucosa. In the 70-year-old population study mucous membrane changes (denture stomatitis, hyperplasia of the mucosa) of the upper jaw were found in 71 per cent of those with a complete denture. A lower frequency of these changes was reported from the lower jaw. Inflammatory changes were found more frequently in women than in men ($p < 0.01$). Among probands without teeth females had a higher frequency of ragades than males ($p < 0.05$).

Clinical findings at the bite physiological examination indicate that disturbances of the bite function are common in 70-year-olds. Eighty-five per cent of males and females showed symptoms of disturbances of the

In spite of the high frequency of disturbances of the bite function the movement capacity of the lower jaw was surprisingly good in the 70-year-olds. The mean values of the movements, the maximum ability of opening the mouth and the side gliding were in those with teeth in the same order of magnitude as in younger individuals (1)

In order to describe the function and the degree of bite disability of the examined 70-year-olds we have used an index referring to four supporting zones of the sides of the jaws. According to this index 14 per cent of those with teeth had a slight bite disability while on the other hand 51 per cent had strongly mutilated bites. When the classification was made with regard to the already performed oral rehabilitation by prothetic means out of all 70-year-olds toothless as well as with teeth 9 per cent had no supporting zones of the bite and had thus a serious functional disability of the bite (37)

By analyses of serum digoxin concentrations the relevance of the interview answers concerning digitalis consumption was evaluated (23). The results indicated that the information concerning at least this drug treatment was adequate. Seventyseven per cent of the females and 61 per cent of the males were on treatment with drugs and 9 per cent had 8 or more different drugs (Figure 3) (24). One woman had been prescribed 24 different drugs, thereof 17 for consumption daily. Every third woman was treated with sedatives and/or sleeping pills and 3 out of 10 women were undergoing treatment with diuretics. The most common drugs were hypnotics-sedatives-atharactica, diuretics, analgetics and vasodillatantia. Eight per cent of the male probands and 12 per cent of the females were aware of allergic reactions to one or more drugs.

Per cent

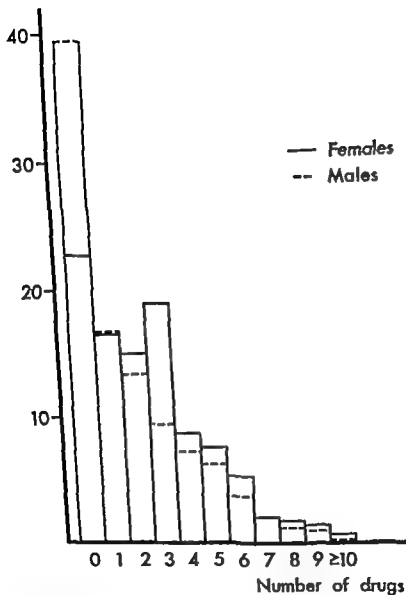


Figure 3 Percentage of probands with different numbers of drugs

Table II Laboratory analyses

		Males		Females	
		M	SD	M	SD
B-Hb	g/l	149	13.4	139	11.2
B-Erythrocytes-volume fraction (hematocrit)	%	43	3.9	41	2.8
B-Erythrocyte sedimentation rate (ESR)	mm/h	12	12.7	15	11.2
B-Glucose	mmol/l	5.66	1.512	5.54	1.630
P-Cobalamines (vitamin B ₁₂)	µmol/l	211	100.3	233	102.0
B-Folate	nmol/l	193	87.6	184	81.4
P-Folate	nmol/l	104	41.2	104	40.3
P-Bilirubin	µmol/l	8.5	3.57	8.5	2.55
P-alkaline phosphatases (37°C)	µkat/l	2.8	1.64	2.8	0.88
P-aspartate-amino-transferase (37°C)	µkat/l	0.34	0.182	0.34	0.216
P-alanine-amino-transferase (37°C)	µkat/l	0.25	0.160	0.22	0.226
P-sodium	mmol/l	140	2.2	140	2.0
P-potassium	mmol/l	3.8	0.37	3.7	0.40
S-protein	g/l	79	6.1	79	5.3

	Males		Females	
	N	SD	N	SD
S-creatinine	88	20.2	79	20.2
S-urea	6	3.7	6	2.4
S-calcium	240	1.30	240	1.15
S-phosphate	0.9	0.16	1.0	0.17
S-iron	18	6.9	17	5.8
S-total iron-binding capacity (TIBC)	65	11.8	66	11.1
S-urate	306	81.0	276	85.2
P-cholesterol	61	1.19	67	1.34
P-triglycerides	1.6	0.84	1.5	0.62
P-total phospholipids	3.37	0.529	3.73	0.564
P-lecithin % of total phospholipids	70.1	5.48	70.1	2.07
P-lysolecithin % of total phospholipids	5.7	1.19	5.0	0.84
P-sphingomyelin % of total phospholipids	21.0	2.50	22.0	2.15
P-cephalin % of total phospholipids	2.7	0.61	2.8	0.53
P-free fatty acids	0.17	0.085	0.21	0.089

Table II Laboratory analyses

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		M	SD	M	SD
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B-Erythrocyte sedimentation rate (ESR)	mm/h	12	12 7	15	11 2
B-Glucose	mmol/l	5 66	1 512	5 54	1 630
P-Cobalamines (vitamin B ₁₂)	pmol/l	211	100 3	233	102 0
B-Folate	nmol/l	193	87 6	184	81 4
P-Folate	nmol/l	104	41 2	104	40 3
P-Bilirubin	μmol/l	8 5	3 57	8 5	2 55
P-alkaline phosphatases (37°C)	μkat/l	2 8	1 64	2 8	0 88
P-aspartate-amino-transferase (37°C)	μkat/l	0 34	0 182	0 34	0 216
P-alanine-amino-transferase (37°C)	μkat/l	0 25	0 160	0 22	0 226
P-sodium	mmol/l	140	2 2	140	2 0
P-potassium	mmol/l	3 8	0 37	3 7	0 40
S-protein	g/l	79	6 1	79	5 3

Table III. Frequency and causes of out-patient consultations (per cent)

Out-patient care consumption (3-year-period):

	<u>Males</u>	<u>Females</u>
> 1 time per month	1 8	2 9
1-2 times per quarter	17 5	25 3
1-3 times per quarter	30 5	39 1
< 1 time per year	31 4	22 0
never	18 8	10 7

Cause of latest out-patient call

Requested routine control	52 3	67 0
First time acute illness	32 3	16 9
Impairment of earlier known illness	12 6	8 9
Health control	2 9	7 2

The results of certain laboratory analyses (performed in in collaboration with Dr Rudolf Jagenburg) are shown in Table II. Certain results of lipid analyses have been published separately (34). The results of laboratory analyses will be published separately more in detail.

The psychological studies were performed by two psychologists, Egil Andersson and Maria Lawenius. The results of tests of vocabulary ability, ability in drawing logical conclusions by the use of reasoning and figure classification and test of sensory-motor speed and coordination as well as immediate memory ability, memory of figures, have been preliminary reported previously (3). Raven's coloured progressive matrices, described as a test of observation and clear thinking and often used in psychogeriatrics, were also used and normative data from this 70-year-old urban population was thus obtained. The psychological findings will be published in detail separately. A general conclusion from these tests is that the results do not indicate any marked reduction of these cognitive functions at the age of 70 with one exception, namely that the sensory-motor speed was markedly lowered at that age.

Table III shows the frequency of out-patient consultations and the reason to the consultation. Every fourth 70-year-old proband apparently visits a doctor 4 or more times a year. Women reported a higher frequency of consultations than men and also that they because of ordained controls went more often than men. On average 6 per cent of the 70-year-olds had visited a physician during the preceding year.

Table III Frequency and causes of out-patient consultations (per cent)

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DISCUSSION

The observations showing such a stable population in Gothenburg with low migration and immigration rates were of course of extreme importance for the evaluation of the results of this study. Certain studies of younger age groups of this population have previously been performed (4 19 35) and will allow certain cross-sectional comparisons between age samples as similar examination methods were used.

The information obtained in conjunction with home calls was of great interest not only from a sociological point of view but also as background data for the medical gerontological as well as the clinical medical examination of this 70-year-old population sample. The 2 nurses who performed the home visits found 94 per cent of the probands that accepted a home call very willing to cooperate, 5 per cent indifferent and only 5 men and 2 women reluctant.

About every sixth woman was living in a situation of loneliness, physical and intellectual inactivity to such an extent that it could have influence upon basic physical and mental functions. Fortyfour per cent of males but only 18 per cent of females go out without hesitation after dark. And daylight time is short in Sweden during half of the year. The fact that every sixth woman declared that one or more days could pass without contact with others is another example on social isolation. The consumption of psychopharmacological drugs was significantly higher in this group than in the others. Those complaining of loneliness significantly more often declared that they did not feel fit but the prevalence of diseases known to influence mobility such as chronic bronchitis and anginal is not overrepresented among those who felt

lonely The lonely women visited physicians more often than the other women These observations seem to indicate that social isolation and physical inactivity is one explanation for the sex difference in consumption of medical care

It should also be emphasized that the detailed penetration of the social background gave in general information that was also of importance with the evaluation of the need of and possibilities of prophylactic measures in this age group The evaluation of the present material concerning possibilities for prophylactic and preventive measures in this age group will be published separately

Fortyeight per cent of the 70 year-olds said that their only hobby was reading but only about 15 per cent had enough light for reading at the location where they usually sit when reading This exemplifies again that the living conditions of the elderly in their present home situation often forces inactivity upon them Inadequate illumination must furthermore mean increased risk of accidents The result of the illumination measurements is only one example on the lack of adjustments of dwellings to the real need of the elderly

Fortytwo per cent of the probands had access to summer cottages And it was obvious that they left their well equipped and usually well functioning flat in the city and moved to less well equipped houses in the country as soon as daylight became longer i.e. usually in April or May and did not return to the city until in October Apparently some of them found a more meaningful activity like gardening and often also an atmosphere with better personal contacts in the rural environment

At the present state of this longitudinal study only limited conclusions can be made concerning incidence

and prevalence of disease in this age group. In many cases ageing per se causes signs very similar to symptoms of disease. More adequate conclusions demand longitudinal studies. A detailed study has however already been tried out in the group of probands treated with digitalis. The results indicate that at least a third and possibly 50-60 per cent of these were on digitalis treatment because of signs that could have been due to normal ageing and were lacking symptoms on definable heart disease (23).

In Sweden the life expectancy at birth for males is about 73 years. Women live longer (about 77 years) than men and one might therefore anticipate that they should be healthier and need less medical care than men. But women consume more drugs and consult physicians more often than men. As far as institutional care is concerned one reason for this sex difference is obviously that it is easier for a man to find care resources at home than a woman. But as far as drug consumption and out-patient care is concerned another explanation must exist. At the age of 70 urinary incontinence, bacteriuria and rheumatic diseases were more common among females but respiratory diseases, cardiovascular diseases, cancer and liver diseases more common among males. Males were thus to a greater extent suffering from life-threatening diseases which within a shorter time can be fatal. The males were however apparently also suffering from a great many less serious diseases and a global judgement of the infirmity of the 70-year-olds does not indicate that the higher consumption of medical care of women is indicated by higher incidence or prevalence of diseases among 70-year-old women.

The present study indicates that overdiagnosis is rather common in this age group due to the fact that the border - or the border zone - between normal ageing and disease is only a very vague presentiment.

in the higher age groups. The author of this report has some difficulty in accepting that 48 per cent of 70-year-old women really should suffer from hypertensive disease. The distribution curves for systolic and diastolic pressure were skewed to the right but without obvious separation of a hypertensive group. At the age of 70 the average systolic and diastolic blood pressures were higher in females than in males; but this does not necessarily mean that the females in the higher age groups suffer more often from a hypertensive disease indicating treatment. The diagnosis of hypertension might be one example of overdiagnosis occurring to women more often than men and therefore to a certain extent explaining the higher consumption of drugs and medical service by women.

The present result also showed underdiagnosis, i.e. that previously unknown diseases often were found at this health survey. During the first two stages of investigation in the out-patient department the general clinical examination and the chemical laboratory test on average one observation generally considered to indicate a disease was discovered per proband and this number was of course further increased during the other stages of the examinations. On average only about 25 per cent of definable diseases observed were previously known to the probands. The preliminary conclusions thus indicated that both overdiagnosis and underdiagnosis are common in this age group.

The observations in the present study showed that only about 3 per cent of the 70 year-olds suffered from advanced handicaps or disease to such an extent that care within institutions was necessary. The need for institutional care shows obviously an acceleration at first in higher age groups. These facts have not been taken into consideration in planning of care for the elderly which is still usually calculated along a line based on the rate of incline of the 65-70 age group.

group It is of importance to clarify which age line shows the change in the average care consumers in the higher age groups of such approximative calculations

Numerous studies of the state of health as well as of social living conditions of the elderly have been performed in different parts of the world (2 4 5 6 7 9 10 11 12 13 14 16 17 18 19 21 22 25 26 28 30 33 35 36) To our knowledge very few epidemiological studies of elderly populations have however been performed with a basic epidemiological technique that really allows generalizations from the investigated sample to larger sections or to well definable sections of the population A general statement out of a comparison of the present observations with those of other population studies is that the social living conditions of the elderly in Europe seem to be rather similar in at least Scotland (17) Norway (14) and Sweden Observations performed in Finland (33) Norway (14) Scotland (17) and Denmark (30) also indicate that the general evaluation of the state of health and frequency of handicaps in the 70-year-old population in Gothenburg Sweden should not be too different from that observed in other studies performed in European countries with similar climatic and general living conditions

It is obvious that the elderly at least in the rural areas of the industrialized countries are rather commonly living under social conditions that from a medical point of view should be subjected to criticism The elderly often suffer from an unmet need of medical support - a need comprising both preventive actions and medical treatment It should however also be emphasized that the majority of the 70-year-olds in Gothenburg are living a very comfortable life in rather good physical and mental condition and seem to have wonderful opportunities of enjoying life

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INTAKE OF ENERGY AND NUTRIENTS AND MEAL HABITS
IN 70-YEAR-OLD MALES AND FEMALES IN GOTHENBURG ; SWEDEN
A POPULATION STUDY

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ABSTRACT

The objectives of this substudy within the population study 70-year-old people in Gothenburg Sweden were to survey the dietary habits of the probands including meal habits and intake of energy and nutrients. The subsample comprised 191 males and 199 females of whom 182 males and 188 females took part in the complete dietary interview examination comprising a 24-hour recall interview and a dietary history interview. The examined subsample was considered representative of the 70 year-old population in Gothenburg. The dietary

well-known decreasing number of cells in the organs is not settled. Favourable to the first-named hypothesis is the fact that isolated mitochondria from hearts of old rats have lower respiratory activity (18) and cytochrome content (5). If this is the case it might be due to a decreased intake of nutrients at higher ages and the subsequent adaptation of the cell or a result of a decreased cell metabolism due to ageing per se. If so - this may mean a decreased need of a supply of nutrients during basal conditions in relation to body cell mass in the elderly individual compared with the need in younger age groups. It may also be claimed that changes in cells and organs may lessen the ability for uptake of nutrients and that this may be compensated for by higher blood concentration of nutrients in the supplying blood. In favour of the last-mentioned hypothesis might be the fact that the homeostasis of some nutrient fractions in blood is changed in such a way that the concentrations increase in higher ages e.g. blood glucose.

There are on the other hand several facts indicating that the main reason for the lower oxygen uptake in elderly individuals is the loss of metabolizing tissue (47) and there are animal experiments which have failed to find any reduction in oxygen uptake of tissue slices, homogenates or isolated mitochondria from rat heart, liver or kidney (11) thus contradicting the above-mentioned studies (5, 18).

There is an obvious need for more knowledge concerning the requirement of energy and nutrients in relation to body cell mass and energy output in elderly people. In a survey of social and medical conditions of 70-year-old people in Gothenburg the design and procedure of which has earlier been presented (46) an attempt has been made to illustrate nutritional questions in one of the older age groups in which many individuals are still capable of an approximately similar average

history method was found to be more valid than the 24-hour recall method. On an average 1.8 hot meals and 0.9 meals of type beverages and sandwich with slices of meat, cheese etc. were consumed daily. The intakes of energy and nutrients were on average satisfactory but showed a fairly great variation. Examples of intakes calculated from the dietary history interview are (M \pm SD, males and females respectively): Energy 9.8 \pm 2.4 MJ and 8.1 \pm 2.3 MJ, protein 74 \pm 18.7 g and 63 \pm 16.5 g, calcium 1033 \pm 414 mg and 927 \pm 368 mg and potassium 79 \pm 22.2 mmol and 68 \pm 25.1 mmol respectively. Examples of significant associations between social data and data of the intake of energy and nutrients are that probands with an education higher than elementary school showed a higher proportion of energy intake from protein than other probands and that males living alone showed a lower iron intake than other males. A comparison between this study and cross-sectional studies on e.g. middle-aged women in Gothenburg showed that the energy intake and the body composition in females was of the same order of magnitude in these two groups. More definite conclusions concerning possible changes by age in intake of energy and nutrients presuppose however longitudinal studies. The present cross-sectional data will be used as a basis for a prospective longitudinal geriatric/gerontological study.

INTRODUCTION

There is reason to believe that aged organs have a decreased metabolism. To what extent this reflects a lower capacity of the single cell and/or just the

well-known decreasing number of cells in the organs is not settled. Favourable to the first-named hypothesis is the fact that isolated mitochondria from hearts of old rats have lower respiratory activity (18) and cytochrome content (5). If this is the case it might be due to a decreased intake of nutrients at higher ages and the subsequent adaptation of the cell or a result of a decreased cell metabolism due to ageing per se. If so - this may mean a decreased need of a supply of nutrients during basal conditions in relation to body cell mass in the elderly individual compared with the need in younger age groups. It may also be claimed that changes in cells and organs may lessen the ability for uptake of nutrients and that this may be compensated for by higher blood concentration of nutrients in the supplying blood. In favour of the last-mentioned hypothesis might be the fact that the homeostasis of some nutrient fractions in blood is changed in such a way that the concentrations increase in higher ages e.g. blood glucose.

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daily physical activity as common in middle-aged people

The study comprises dietary interviews the relevance of which has been checked by analyses of urinary excretion of nitrogen. The study furthermore includes other data of nutritional interest such as studies of body composition with isotope techniques and studies of the relation between oral health and nutritional data. These studies will be reported in subsequent papers (29-50) whereas the present paper is the first in this series concerning nutrition of the investigated group.

In this paper the relevance in this age group of the dietary interview methods used will be discussed. Dietary habits and intake of energy and nutrients will be presented and the intake compared to existing standards.

MATERIAL

The population study 70-year-old people in Gothenburg comprised a representative systematic sample of 1148 70-year-old people in Gothenburg which was obtained from the Revenue Office Register in a way described previously (46). The subjects were numbered consecutively (1 2 3 4 5 1 2 etc.) to make subsampling possible.

Probands 3 and 4 who took part in the hospital part of the study (191 males and 199 females) were invited to participate in a dietary interview. It was pointed out that this interview was an important complementary

part of the examination. It was arranged at the hospital for a dietitian to visit the probands in their own homes some time after the hospital examination.

A dietary interview was completed in 370 probands: 182 males and 188 females (24-hour recall and dietary history) and 4 probands: 1 male and 3 females (24-hour recall only). Non-responders from the dietary history interview were 20 probands (9 males/4.7 per cent/ and 11 females/5.5 per cent/). The reasons for this non-response were refusal and incooperability.

In order to test the representativity of the dietary history interviewed probands, they were compared with the other probands in the study regarding sex, marital status, proportion of males registered in the Register of Temperance Board, income and community rent allowances. No significant differences ($p > 0.05$, χ^2 -test and Student's *t*-test respectively) in this register data were observed. As far as height, body weight, waist girth, triceps and subscapular skinfold, B-Hemoglobin, B-Glucose, P-Triglycerides and S-Iron were concerned, only one significant difference between this subsample and the other probands was observed on the one per cent level (Student's *t*-test), namely a difference in triceps skinfold thickness in the females (Table I). Our general conclusion is therefore that the subsample did not differ in physical characteristics.

METHODS

As a first part of the hospital examination in the morning and in a fasting state, blood samples were

drawn (with Vacutainer[®] technique) from an antecubital vein without stasis and with the proband in the sitting position. Blood was collected in one 5 ml EDTA glass tube used for determination of 1 a B-Hemoglobin in 10 ml heparin tubes which were immediately centrifuged and in 10 ml glass tubes where the blood was left to clot for three hours at room temperature and then centrifuged. Plasma and serum which were not analysed fresh were stored at -18°C in sealed glass ampoules. Capillary blood was collected from the finger tip for estimation of blood glucose level.

B-Hemoglobin was determined with the methemoglobin-cyanide method and B Glucose with the o-toluidin method described by Härtel et al (32). S-Iron and S-Total iron binding capacity (TIBC) were determined with autoanalyzer technique using conventional methods. P-Cyano-cobalamines (Vitamin B₁₂) were determined with the microbiological method of Hutnar et al (31) using *Euglena Gracilis* (1-strain). P-Folate was assayed with *Lactobacillus casei* according to a modification of the technique described by Hansen (28). P-Potassium was determined using conventional flame photometry. Total P-Protein was determined with a standard biuret method. P-Cholesterol was determined with the gas-liquid chromatographic method described by Blomhoff (13) and modified by Lillienberg and Svanborg (35) and S-Tri-glycerides according to Carlson and Wadström (17). Skinfold measurements were performed with a Harpenden caliper (21) with a pressure of 10 g/cm^2 applied at a contact surface of 20 mm^2 . The skinfold was measured at the back of the right upper arm midway between acromion and elbow with the arm hanging relaxed and with the fold running parallel to the length of the arm - subject standing - (triceps skinfold) and just below the angle of the right scapula with the fold parallel to the natural cleavage line of the skin -

subject standing - (subscapular skinfold) Waist girth was measured horizontally half-way between the lowest floating ribs and iliac crests - subject standing

The dietary interviews were performed in the probands own homes on an average of 23 days after the hospital examination. The interview was carried out on 242 probands (65 per cent) within two weeks and on 276 probands (74 per cent) within four weeks after the hospital examination. The longest interval was 191 days for one proband due to the fact that this proband was not at home at the time originally agreed upon and thereafter was difficult to get in touch with.

The same dietitian (Inga-Lill Landin) interviewed all the probands. The interview was performed according to the 24-hour recall method, the principle of which was originally introduced by Wiehl (56) and the dietary history method originally described by Burke (15).

As stipulated in the 24 - h o u r r e c a l l method the probands were interviewed about their food consumption during the last 24 hours. They were asked about the number and distribution of meals throughout these 24 hours and the composition of the meals. The classification of the meals used is described in Table II.

The choice of food items is described in terms of 7 food groups in accordance with the usage of the Swedish National Food Administration (former National Institute of Public Health) - see (57)

- green vegetables
- fruit/berries
- potatoes/other starches
- milk products
- meat/fish/eggs
- bread/other flour products
- fats/oils for cooking and spreading

Table II Different types of meals

M	Hot meals: Cooked and fried dishes
S ₁	Beverages: Lemonade juice coffee tea beer wine clear soup Sandwiches with slices of meat cheese etc
S ₂	Beverages: see S ₁ Biscuits cakes buns pastry etc plain bread and butter
S ₃	Milk: all kinds of milk cocoa cream fermented milk Sandwiches with slices of meat cheese etc
S ₄	Milk see S ₃ Biscuits cakes buns pastry etc Cornflakes ³ Plain sandwiches
S ₅	Cannot be classified as above e.g. beverages only (a plain cup of coffee/tea was however not noted) Sweetmeat raisins desserts only and fruit only

Furthermore data on consumption of alcoholic beverages sugar and sweet meat is given

The probands were asked if their 24-hour recall was given regarding a representative day The probands also answered questions of socio-dietary interest e.g. facilities for cooking and storage of food

The dietary history method is aimed to illustrate the ordinary diet of the probands - as was pointed out to them A questionnaire especially adopted to this material was used For each food item the probands were asked about the frequency of consumption of that particular item and also about the size of the portion A cross-check of the answers was made throughout the interview One example of such cross-checking - after a detailed penetration of the number of sandwiches with different kinds of bread with or without slices of meat cheese etc - was to check that the number of sandwiches so obtained was the same as the number given earlier in the interview as an answer to the general question: How many sandwiches a day do

you usually eat? Another example was checking that the daily number of claimed lumps of sugar in cups of coffee corresponded to the earlier claimed daily number of cups of coffee To facilitate the estimation of the size of portions models of food articles were shown to the probands in some cases

The intake of energy and nutrients was then calculated using a computer system worked out at the Department of Clinical Nutrition University of Gothenburg (10) The system is mainly based on data published by Abramson (4)

Contrary to the 24-hour recall method the data from the dietary history method does not include information about the consumption of wine and liquor (Beer consumption is however included) The consumption of wine and liquor was asked for separately in a more general way at the end of the other interviews in order to minimize the risk of poor cooperation The results of the dietary history interviews below do not include this data which is reported separately However in about 3 per cent of males (because of wine consumption) and in about 10 per cent of males (because of liquor consumption) an underestimation of the energy intake must be suspected because of this Such an underestimation is on average neglectable in females

In order to investigate possible differences throughout the year regarding the calculated intake of energy and nutrients an intermonth analysis of variance and a comparison between the period April to September and the period October to March respectively were performed Since the primary interest in these analyses was focused towards differences between the periods rather than the absolute levels of intake and since the dietary history method (which aims to describe the ordinary diet of the proband) can be expected not to reveal to the same extent such possible seasonal

differences the results of the 24-hour recall method were used in these analyses

In order to study the association of some social and medical variables with data of intake of energy and nutrients a series of hypotheses was tested. Thus contrast groups were studied regarding 14 dietary variables (Table III). The contrast groups were either dicotomic (e.g. living alone - not living alone) or extreme groups (e.g. upper quartile - lower quartile of a continuous laboratory variable). Thus 27 pairs of contrast groups were studied with respect to intake of energy and nutrients i.e. altogether 378 hypotheses. The differences between the contrast groups were tested with Student's t-test or - in the case of small groups - with the Mann-Whitney U-test. In these studies of possible associations of certain social and medical variables with nutrition data only test results significant on the one per cent level are reported. When such significance was observed in only one sex significant results on the 5 per cent level are also given for the other sex.

Table III Dietary intake variables (dietary history method) tested in the hypotheses (see text)

Energy
Protein
Proportion of energy from protein
Fat
Proportion of energy from fat
Carbohydrates
Proportion of energy from carbohydrates
Calcium
Iron
Potassium
Vitamin A
Thiamin
Riboflavin
Ascorbic acid

you usually eat? Another example was checking that the daily number of claimed lumps of sugar in cups of coffee corresponded to the earlier claimed daily number of cups of coffee. To facilitate the estimation of the size of portions, models of food articles were shown to the probands in some cases.

The intake of energy and nutrients was then calculated using a computer system worked out at the Department of Clinical Nutrition, University of Gothenburg (10). The system is mainly based on data published by Abramson (4).

Contrary to the 24-hour recall method, the data from the dietary history method does not include information about the consumption of wine and liquor (Beer consumption is however included). The consumption of wine and liquor was asked for separately in a more general way at the end of the other interviews in order to minimize the risk of poor cooperation. The results of the dietary history interviews below do not include this data which is reported separately. However, in about 3 per cent of males (because of wine consumption) and in about 10 per cent of males (because of liquor consumption) an underestimation of the energy intake must be suspected because of this. Such an underestimation is on average neglectable in females.

In order to investigate possible differences throughout the year regarding the calculated intake of energy and nutrients, an intermonth analysis of variance and a comparison between the period April to September and the period October to March, respectively, were performed. Since the primary interest in these analyses was focused towards differences between the periods rather than the absolute levels of intake and since the dietary history method (which aims to describe the ordinary diet of the proband) can be expected not to reveal to the same extent such possible seasonal

Table IV Intake of energy and nutrients calculated with 24-hour recall method (24-R) and with dietary history method (DH) in those probands (182 males and 108 females) who participated in both interviews M mean value SD standard deviation

	Males				Females				Average	
	24-R		DH		24-R		DH		24-R value in per cent of average DH value	
	M	SD	M	SD	M	SD	M	SD	DH value	DH value
Energy (kcal)	2121	590.0	2344	574.3	90.5	1672	548.9	1928	545.3	86.7
(MJ)	8.9	2.5	9.8	2.4		7.0	2.3	8.1	2.3	
Protein (g)	67	20.3	74	18.7	90.5	56	18.5	63	16.5	88.9
Fat (g)	88	30.3	96	32.3	91.7	69	27.9	80	30.1	86.3
Carbohydrates (g)	250	79.8	279	73.4	89.6	198	68.8	227	73.8	87.2
Calcium (mg)	789	394.1	1033	414.0	76.4	718	343.8	927	368.0	77.5
Iron (mg)	14.1	4.82	16.5	4.17	85.5	11.7	5.00	14.0	4.37	83.6
Potassium (mmol)	65	20.6	79	2.2	82.3	56	17.2	68	25.1	82.4
Vitamin A (μ g retinol)	956	684.2	1505	601.5	63.5	899	1034	1357	684.2	66.2
Thiamin (mg)	1.3	0.53	1.4	0.35	92.9	1.0	0.40	1.2	0.43	83.3
Riboflavin (mg)	1.4	0.63	1.8	0.61	77.8	1.3	0.78	1.6	0.54	81.3
Ascorbic acid (mg)	73	47.2	82	41.7	89.0	80	52.5	87	53.7	92.0

On the visit by the dietitian to the probands homes oral and written information was given regarding the sampling of a 24-hour urinary volume from 7 a m the following morning The urine was then collected by the dietitian on a second visit and then frozen A report was written out with data of the dietitian s judgement of the reliability of the urinary sampling On the basis of this report urine volumes were judged as adequate or not Urinary nitrogen was analyzed using the Technicon Autoanalyzer Method N-3b The average non-urinary loss of nitrogen was estimated to be 2 0 g of nitrogen per day (10)

In the statistical analyses χ^2 -test Student's t-test the Mann-Whitney U-test and analyses of variance were used The method used is referred to in the text In situations when many variables were tested and the mass significance problem thus was actual results were considered significant only at the one per cent level in this report

RESULTS

Dietary history versus 24-hour recall

A comparison of the results of the two methods used for the calculation of the intake of energy and nutrients was made in those probands in whom both methods were used (Table IV) The average 24-hour recall values were constantly lower than the dietary history values The average calculated intake of energy protein fat and carbohydrates was with the 24-hour recall method about 90 per cent of the dietary history values but

energy and nutrients and the description of the number and kind of meals throughout the day where the 24-hour recall results were used

Number of meals

The probands had - from the dietary history - on average 4.5 ± 0.99 (males) and 4.3 ± 0.79 (females) meals a day ($M \pm SD$) with a range of 1-7 (males) and 3-6 (females). The daily number of hot meals was in no male and 1 female no hot meal in 48 males and 51 females one hot meal in 12 males and 123 females two hot meals and in 9 males and 13 females three hot meals.

The results below regarding the numbers and kinds of meals throughout the day are taken from the 24-hour recall interview with those probands who claimed their 24-hour recall to refer to a representative 24-hour period (77 per cent of males and 75 per cent of females). The number of meals are given in Table V which shows that on average 2.7 meals of the types hot meals (M) and beverages and sandwiches with slices of meat cheese etc (S_1) were consumed daily.

Table V Average daily number (M) of different types of meals (see Table II) per proband SD: standard deviation

Type of meals	<u>Males (n=141)</u>		<u>Females (n=142)</u>	
	M	SD	M	SD
M	1.8	0.56	1.8	0.72
S_1	0.9	0.79	0.9	0.81
S_2	1.2	0.89	1.1	0.84
S_3	0.1	0.50	0.3	0.43
S_4	0.1	0.30	0.1	0.29
S_5	0.1	0.28	0.1	0.39

even greater differences were observed in the calculation of intake of certain nutrients. As far as intake of vitamin A was concerned the 24-hour recall method only revealed about 65 per cent of the dietary history values. The correlation coefficients between data obtained from the two methods varied between $r=0.66$ (energy females) and $r=0.26$ (fat males). All coefficients differed significantly ($p<0.001$) from 0.

The validity of the two interview methods was tested by a comparison of the intake of protein calculated from the interviews and from the results of analyses of nitrogen in 24-hour urinary samples in those 349 probands (172 males and 177 females) in whom the urinary sampling was considered adequate. The 24-hour urinary excretion of nitrogen was 10.2 ± 3.55 in males and 8.6 ± 2.31 g in females ($M \pm SD$).

When the intake of protein was calculated from the sum of the average urinary nitrogen excretion values and the estimated non-urinary losses, an estimated average total nitrogen loss of 12.2 g (males) and 10.6 g (females) was obtained. The corresponding protein figures (nitrogen figure multiplied by 6.25) were 76 g (males) and 66 g (females). These values are very similar to and not significantly ($p>0.05$, Student's t-test) different from those obtained by using the dietary history method (74 g and 63 g respectively) but significantly higher ($p<0.001$, Student's t-test) than the interview values obtained using the 24-hour recall method (67 g and 56 g respectively).

These studies of the validity of the interview methods strongly support the conclusion that the dietary history method had given the most valid results. In this report only results of the dietary history method are reported below with two exceptions, namely the analysis of possible seasonal variation in intake of

energy and nutrients and the description of the number and kind of meals throughout the day where the 24-hour recall results were used

Number of meals

The probands had - from the dietary history - on average 4.5 ± 0.99 (males) and 4.3 ± 0.79 (females) meals a day ($M \pm SD$) with a range of 1-7 (males) and 3-6 (females). The daily number of hot meals was in no male and 1 female no hot meal in 48 males and 51 females one hot meal in 125 males and 123 females two hot meals and in 9 males and 13 females three hot meals

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S_2	1.2	0.89	1.1	0.84
S_3	0.3	0.50	0.3	0.43
S_4	0.1	0.30	0.1	0.29
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Choice of food groups in the different types of meals

Table VI illustrates the proportion of the three most common kinds of meals in which different food groups were included

About one third of the hot meals contained green vegetables and about one fourth fruit/berries. There was a slight female dominance for both of these food groups. Potatoes/other starches were included in only half of the hot meals. The majority of hot meals included milk products and the same was true for meat/fish/eggs and bread/other flour products. A little more than half of the hot meals included fats for cooking and/or spreading. Thirtyseven per cent of hot meals in males and 39 per cent in females included more than four food groups (Table VII)

Table VII Percentage of hot meals containing different number of food groups

Number of food groups	Males	Females
1	1	1
2	7	11
3	19	16
4	37	34
5	25	26
6	10	10
7	2	3

Seventyfive per cent (males) and 88 per cent (females) of the S₁ type of meals included milk products. Slightly less than half of these meals included meat/fish/eggs

Table VI Percentage of hot meals (M) meals including beverages and sandwiches with slices of meat cheese etc (S₁) and meals including beverages and buns etc or beverages and sandwiches without slices of meat cheese etc (S₂) which included different food groups

	H	S ₁		S ₂		
		Males	Females	Males	Females	
Green vegetables	31	39	7	8	1	0
Fruit/berries	20	27	7	9	3	3
Potatoes/other starches	52	56	2	2	0	1
Milk products	70	80	75	84	32	39
Meat/fish/eggs	89	84	48	44	0	0
Bread/other flour products	82	82	100	100	100	100
Fats	62	52	96	98	27	24

Table VIII Average daily number (M) of different types of meals (see Table II) per proband in those interviewed probands (P) belonging to upper quartile of the /difference between body weight and ideal body weight (36)/ divided by body weight and other interviewed probands (NP) SD standard deviation

Males				Females					
Type of meals		P (n=35)		NP (n=106)		P (n=33)		NP (n=109)	
	M	SD	M	SD	M	SD	M	SD	
M	1.8	0.51	1.8	0.58	1.7	0.64	1.8	0.70	
S ₁	0.8	0.68	0.9	0.82	0.8	0.71	0.9	0.85	
S ₂	1.2	0.79	1.2	0.91	1.1	0.68	1.1	0.88	
S ₃	0.1	0.36	0.3	0.48	0.2	0.39	0.3	0.44	
S ₄	0.1	0.24	0.1	0.30	0.1	0.24	0.1	0.31	
S ₅	0.1	0.28	0.1	0.28	0.2	0.39	0.1	0.40	

and only a few per cent potatoes/other starches : These meals contained green vegetables in less than ten per cent and the same applied fruit/berries (Table VI)

Daily meal pattern

The distribution throughout the day of the 3 most common types of meals (M, S_1 and S_2) is given in Figures 1-2 Hot meals were more often consumed at dinner time than at lunch time Meals of types S_1 and S_2 were distributed more evenly throughout the day

In order to elucidate if obese probands had a different daily number of meals and/or different distribution of meals throughout the day the meal habits of probands belonging to the upper quartile of /the difference between body weight and ideal body weight (36)/ divided by body weight in the total material were compared with the meal habits of the rest of the material A rather good correlation ($r=0.71$ males and $r=0.91$ females) has been found in a subsample of this material between the above mentioned difference and body fat calculated from the body composition studies (50) No significant ($p>0.05$ Student's t-test) differences between the obese and the other probands were found regarding the number of the three quite dominating types of meals (namely hot meals and meals type S_1 and S_2) A low-grade ($p<0.05$ Student's t-test) significant difference was however found regarding the number of meals type S_3 where obese males showed fewer such meals The absolute number of these meals was however very small and our general conclusion is therefore that these obese probands generally did not differ from the rest of the material regarding number of meals (Table VIII)

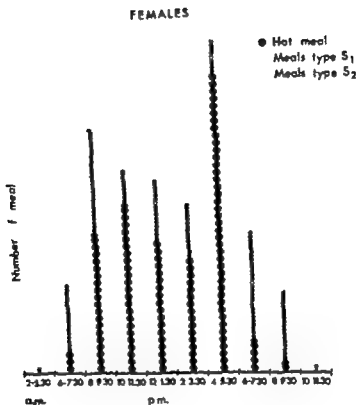


Figure 2 Distribution of hot meals meals including beverages and sandwiches with slices of meat cheese etc (S_1) and meals including beverages and buns etc or beverages and sandwiches without slices of meat cheese etc (S_2) throughout the day (females) Each full symbol represents two females

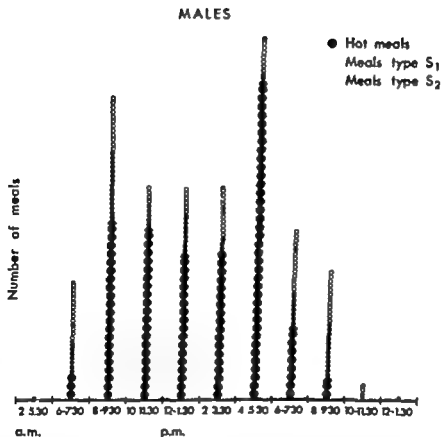


Figure 1 Distribution of hot meals meals including beverages and sandwiches with slices of meat cheese etc (S_1) and meals including beverages and buns etc or beverages and sandwiches without slices of meat cheese etc (S_2) throughout the day (males) Each full symbol ² represents two males

Table IX Storage facilities for food (per cent)

	Males	Females
None	1	1
Larder only	4	3
Refrigerator only	8	10
Larder + refrigerator	56	67
Larder + freezer	1	1
Refrigerator + freezer	4	5
Larder + refrigerator + freezer	27	14

Table X Proportion of answers to the question: Who does usually the cooking? (per cent)

	Males	Females
Proband	21	90
Spouse	72	0
Relative	3	1
Friend	1	1
Domestic help home care for the aged	2	1
Eating out	2	0

Alcoholic beverages

In males daily consumption of beer/lager was reported in 31 per cent of wine in 3 per cent and of liquor in 10 per cent (Table XI). It should be noted that data about wine and liquor consumption is not included in the results from the dietary history interview. Energy data does however include energy derived from beer/lager which however contributes to energy intake in

Regarding the distribution of the different kinds of meals throughout the day there were no obvious differences between the obese group and the other probands regarding hot meals and meals of type S_1 . However in females significantly ($p < 0.01$ χ^2 -test) more meals of type S_2 were eaten before noon than after noon in the obese group compared to the other probands. This difference was not observed in males. Furthermore in 48 per cent of the obese females a meal of type S_2 was their first meal in the morning compared with 26 per cent in other females ($p < 0.05$ χ^2 -test). The proportion of hot meals was the same in the two groups of females. When the meals of types S_1 and S_2 that broke the fast were analysed separately 30 per cent of the meals were of type S_1 and 70 per cent of type S_2 in the obese female group compared to 61 per cent and 39 per cent respectively in the other females ($p < 0.05$ χ^2 -test).

Kitchen facilities and cooking

Practically all probands (99 per cent) had the possibility of cooking regarding access to a kitchen (males 97 per cent females 94 per cent) or kitchenette. About as many probands had an electric cooker (55 per cent) as had a gas-cooker (43 per cent) and only one proband had a wood stove as her only cooking facility. Only a few probands (4-5 per cent) had no refrigerator. 32 per cent of males and 20 per cent of females had a freezer (Table IX). Frozen dishes were bought sometimes or often in less than a fifth of the probands. About 20 per cent of the male probands did their own cooking. In fact these were with few exceptions the male probands living alone (Table X).

Table XII Intake of energy and nutrients calculated with the dietary history method M: mean value SD standard deviation 1st: 1st decentile m median value 9th: 9th decentile

	Males				Females					
	M	SD	1st	m	9th	M	SD	1st	m	9th
Energy (kcal)	2344 ⁺	574.3	1720	2292	2951	1928 ⁺	545.3	1262	1888	2576
(MJ)	9.8	2.4	7.3	9.6	12.4	8.1	2.3	5.3	7.9	10.8
Protein (g)	74	18.7	51	72	97	63	16.5	45	61	85
Fat (g)	96	32.3	63	91	130	80	30.1	47	77	117
Carbohydrates (g)	279	73.4	201	274	366	227	73.8	141	221	315
Calcium (mg)	1033	414.0	514	1021	1632	927	368.0	514	865	1384
Iron (mg)	16.5	4.17	11.7	16.0	21.4	14.0	4.37	9.0	13.3	19.6
Potassium (mmol)	79	22.2	52	78	109	68	25.1	46	65	97
Vitamin A (µg retinol)	1905	801.5	670	1201	2881	1357	684.2	555	1242	2287
Thiamin (mg)	1.4	0.35	1.0	1.4	1.9	1.2	0.43	0.8	1.2	1.7
Riboflavin (mg)	1.8	0.61	1.0	1.8	2.6	1.6	0.54	1.0	1.5	2.3
Ascorbic acid (mg)	82	41.7	30	76	129	87	53.7	34	74	162

⁺ Further daily intake of energy from wine and liquor was reported by about 10 per cent of males and 1-2 per cent of females (Table XI)

Table XI Proportion of answers to the questions Do you drink beer and lager wine and liquor? respectively (per cent)

	<u>Beer/lager</u>		<u>Wine</u>		<u>Liquor</u>	
	Males	Females	Males	Females	Males	Females
Never or a few times a year	40	69	85	87	62	91
Every week but not every day	29	24	12	12	28	9
Every day $\leq 1/2$ bottle	28	7	3	1	8	1
Every day $> 1/2$ bottle	3	0	0	0	2	0

The intake of energy and nutrients - with the exception of Vitamin A and ascorbic acid - was generally higher in probands with 6-7 meals a day than in probands with 1-3 meals a day. Such a difference was not observed when probands with 3 cooked meals a day were compared with probands with 0-1 cooked meals a day - except for energy and iron intake in females (Table XIV).

The intermonth analysis of variance revealed no significant differences on the one per cent level. When the winter half of the year (October-March) was compared with the summer half (April-September) a significant difference was obtained in males regarding the intake of fat ($p < 0.01$) with a higher average value during the winter half of the year. It should be mentioned that possible significant differences ($p < 0.05$) were observed regarding intake of energy and protein with higher average values also during the winter.

In the studies of possible associations of certain social and medical variables with nutrition data the following significant results were obtained (Table XIV). Among the social hypotheses tested probands with an education higher than elementary school showed a greater proportion of energy intake from protein than other probands and the protein proportion of energy and potassium intake was correlated to income in males. Males living alone showed a lower iron intake than other males.

As far as possible correlations between medical history data and dietary intake were concerned differences were only observed in the diabetics (Table XIV). Females with diabetes showed a higher proportion of energy intake from protein than other females and males with diabetes reported a lower intake of carbohydrate and a lower proportion of energy intake from carbohydrates than other males. Probands who claimed they felt healthy did not differ from other probands.

males in an average order of magnitude of only 2 per cent

Sugar and sweetmeat

The average energy intake from lumps of sugar and powdered sugar was 94 kcal/0.4 MJ in males and 53 kcal/0.2 MJ in females. The average energy intake from candies, chocolate and bonbons was 30 kcal/0.1 MJ in males and females. The average proportion of energy from sugar and sweetmeat thus amounted to 5 per cent for males and 4 per cent for females.

Intake of energy and nutrients

Table XII gives the calculated intake of energy and nutrients obtained from the dietary history interview method and Table VIII gives the proportion of energy intake from protein, fat and carbohydrates respectively. An evaluation of the intakes obtained is made in the discussion.

Table XIII. Average proportion of energy intake (M) from protein, fat and carbohydrates respectively (per cent). SD: standard deviation.

	<u>Males</u>		<u>Females</u>	
	M	SD	M	SD
Protein	13.0		13.7	
Fat	37.9		38.4	
Carbohydrates	48.9		48.3	

(Table XIV cont.)

	Contrast groups		Significant results	
	I	II	Males	Females
Examination	Peptic ulcer	Not peptic ulcer		
	Digitalis treatment	Not digitalis treatment		
	Upper quartile of triceps skinfold	Lower quartile of triceps skinfold		
	subscapular skinfold	subscapular skinfold		
	waist girth	waist girth		
	height	height		
	/body weight minus ideal body weight (36)/divided by body weight	/body weight minus ideal body weight (36)/divided by body weight	Protein ^a (xx)	Energy ^b (xx)
			Protein proportion of energy ^a (xx)	protein proportion of energy ^a (x)
Laboratory analyses	Upper quartile of B-Hemoglobin	Lower quartile of B Hemoglobin		
	B-Glucose	B-Glucose		
	S-Iron	S Iron		
	S-TIBC	S-TIBC		

Fat^b (xx)

Table XIV Result of tests of hypotheses (see text) Significant results are given on the 0.1 per cent level (xxx) and the 1 per cent level (xx) - χ^2 -test Student's t-test - and in the opposite sex also on the 5 per cent level a: contrast group I higher value b: contrast group I higher value

	Contrast groups		Significant results	
	I	II	Males	Females
Social interview	Living alone	Not living alone	Iron ^b (xx)	
	Upper quartile of income	Lower quartile of income	Protein proportion of energy ^a , potassium ^a (xx)	
	Registered at Temperance Board	Not registered at Temperance Board		
	Higher education than elementary school	Elementary school	Protein proportion of energy ^a (xx)	Protein proportion of energy ^a (xxx)
History	Feels healthy	Does not feel healthy		
	Diabetes	Not diabetes		Carbohydrate proportion of energy ^b (xxx) carbohydrates ^b (xx)
	Gallstone	Not gallstone		Protein proportion of energy ^a (xxx)

regarding dietary intake. This was also the case in patients treated with digitalis.

Tall male probands had a higher intake of protein than short ones (Table XIV). Female probands with a high ratio /body weight - ideal body weight · (36)/ to body weight reported a lower intake of energy than female probands with a low such ratio and all such probands reported a higher proportion of energy intake from protein than probands with a low such ratio. Female probands with a high triceps skinfold value showed a lower intake of fat than females with a low triceps skinfold value.

Generally speaking dietary intake did not differ in the high and low quartile groups regarding laboratory analyses (Table XIV). However female probands with a high B-Folate value showed a higher intake of vitamin A, ascorbic acid, potassium, thiamin and riboflavin than females with a low B-Folate value. Probands with a high P-Cyano-cobalamines (Vitamin B₁₂) value had a higher proportion of energy intake from protein than probands with a low P-Cyano-cobalamines value. Male probands with a high P-Cholesterol value showed a higher intake of potassium than males with a low P-Cholesterol value.

A comparison of dietary intake of protein, calcium, iron and potassium and their concentrations in blood showed no significant correlations.

(a XIV cont)	Contrast groups		Significant results	
	I	II	Males	Females
	P-Cyano-cobalamines (Vitamin B ₁₂)	P-Cyano-cobalamines (Vitamin B ₁₂)	Protein proportion of energy ^a (xx)	Protein proportion of energy ^a (xx)
	B-Folate	B-Folate	Ascorbic acid ^a (x)	Vitamin B ^a ascorbic acid ^a (xxx) potassium ^a thiamin ^a riboflavin ^a (xx)
	P-Potassium	P-Potassium		
	P-Protein	P-Protein		
	P-Cholesterol	P-Cholesterol	Potassium ^a (xx)	
	S-Triglycerides	S-Triglycerides		
etary interview	Upper quartile of food cost per month	Lower quartile of food cost per month		
	1-3 meals a day	6-7 meals a day	Energy ^b carbohydrates ^b iron ^b thiamin ^b potassium ^b calcium ^b riboflavin ^b (x)	Energy ^b protein ^b carbohydrates ^b calcium ^b iron ^b thiamin ^b riboflavin ^b (xxx) fat ^b potassium ^b fat ^b (xx)
	0-1 hot meal a day	3 hot meals a day		Energy ^b iron ^b (xx)

studies in younger persons possible special dietary habits during Sundays will not have any great influence on the results (10)

Even though there was rather good agreement between the protein intake data obtained with the dietary history method and the intake calculated from urinary nitrogen analyses in the total material it might be suspected that certain groups of individuals e.g. those with diabetes and/or obesity might report dietary habits closer to prescribed diet than their actual food intake. Thus probands belonging to the upper quartile of $\text{body weight} - \text{ideal body weight} (36) / \text{body weight}$ apparently underestimated their dietary intake. Their average protein intake calculated with the dietary history method (74 g and 51 g for males and females respectively) was apparently lower ($p < 0.05$ Student's t-test) than the corresponding figure obtained from calculation from urinary analyses (85 g and 66 g respectively). Probands belonging to the lower quartile of this ratio did not show such a discrepancy. In 12 male patients with earlier known and treated diabetes the average reported protein intake according to the dietary history method was apparently lower (72 g) than the protein intake calculated from urinary nitrogen analyses (85 g $p < 0.05$ Student's t-test). It thus seems that the male diabetics underestimated their dietary intake. In 13 female diabetics the corresponding figures were 66 and 72 g respectively ($p > 0.05$ Student's t-test) but the diabetic females reported a higher ($p < 0.001$ Student's t test) proportion of energy intake coming from protein than other female probands.

There exist numerous reports with recommendations regarding intake of certain nutrients and reference values for energy consumption. In the comparison between the present results and this data our discussion will be mainly restricted to two earlier

The general design of this population study the evaluation of basic epidemiological methods and intra- and interobserver variation studies have been reported earlier (46). That report included an intraobserver variation study of the calculation of the intake of energy and nutrients performed by the dietitian which showed no significant differences ($p > 0.05$ Student's t-test) between the results of calculations of dietary history values from 40 consecutive probands of intake of energy and nutrients performed by the same dietitian on two different occasions. The available data indicates that the sampling techniques and methods used for collection, registration and processing of data were adequate.

As motivated earlier the data obtained from the 24-hour recall method was only used in the analyses of possible seasonal variation in intake of energy and nutrients and in the description of number and kinds of meals. The way the 24-hour recall method was used in the present study can be criticized as no interviews covered the dietary habits on Fridays and Saturdays. It is of course possible that the probands ate more just on Fridays and Saturdays and thus compensated for a lower food intake during the rest of the week. Our results of the comparison of the urinary excretion of nitrogen and the protein intake showed that the correspondance between the dietary history method and the calculations from urinary analyses was good. No urinary sampling was however performed on Sundays and Mondays. The possibility that the 70-year-olds consumed more or less energy during Sundays than during the rest of the week should of course be taken into consideration. However this was not the case in the present material and as far as can be judged from

males very similar average figures regarding calculated energy expenditure (2329 kcal/9.8 MJ) and energy intake calculated with the 2-days record method (2399 kcal/10.1 MJ). The average calculated energy expenditure in females in that study (1943 kcal/8.2 MJ) was close to the figures in this study. The calculated energy intake in the study by Loneragan et al. (37) was however lower (1733 kcal/7.3 MJ). The variation of intake of energy in the present study was fairly great. Thus the upper decentile value exceeded RDA 1974 by more than 500 kcal/2.1 MJ (males) and by more than 700 kcal/2.9 MJ (females). This need not necessarily be interpreted as overeating but as a result of more pronounced physical activity compared with the average 70-year-old person. The lower decentile value was almost 700 kcal/2.9 MJ (males) and more than 500 kcal/2.1 MJ (females) below the RDA 1974.

Protein

As far as the need of dietary protein is concerned Veiledning considers 0.9 g protein per kg body weight a high intake, 0.8 g per kg body weight an acceptable intake and 0.7 g per kg body weight a low/minimum intake. RDA 1974 recommend 0.8 g per kg body weight. According to the figure of theoretical safe level of protein by FAO/WHO (23) the level of dietary protein is lower: 0.57 g protein per kg body weight in males and 0.52 g in women but this statement is valid for proteins like egg and milk proteins. The average protein intake per kg body weight in this study was 0.97 g (males) and 0.95 g (females). The corresponding median values were 0.93 g (males) and 0.92 g (females). Thus on an average the intake of protein was high in the upper decentile groups very high. Female probands in the lower decentile group had however an intake below the low/minimum value of Veiledning. In fact

reports 1 a Veiledning til vurdering og planlægning af kosthold by Eeg-Larsen et al 1971 (22) referred to in the text as Veiledning and Recommended Dietary Allowances 8th Edition by the Food and Nutrition Board 1974 (24) referred to in the text as RDA 1974 Three steps are given in Veiledning (high acceptable and low/minimum) for the intake of nutrients in order to make an evaluation of the diet of adults possible RDA 1974 is "designed for the maintenance of good nutrition of practically all healthy people in the U S A In this context the RDA 1974 values are taken from the data in the group of persons 51 years of age and older

In Sweden the National Food Administration gives recommendations of nutrients per 1000 kcal/4.2 MJ However these recommendations are intended to be used in heterogenous groups of individuals which is the reason why we have chosen to compare the intakes with the data of Veiledning and RDA 1974 in this presentation

Energy

RDA 1974 gives an energy value for males 51 years and over of 2400 kcal/10.1 MJ and for females 1800 kcal/7.6 MJ Veiledning gives similar values for individuals 55-75 years and over (2400 kcal/10.1 MJ and 1700 kcal/7.1 MJ for males and females respectively) These figures thus refer to persons both younger and older than 70-year-olds Furthermore the reference persons in RDA 1974 and Veiledning have a lower body weight than the probands of the present material Our figures are close to those of RDA 1974 and Veiledning regarding males Furthermore Lonergan et al (37) studied the energy expenditure of elderly people according to principles of Durnin and Passmore (20) and found in

Iron

Both Veiledning and RDA 1974 give a recommended iron value of 10 mg. On an average the intake of iron was thus high. The lower decantile value was in males above 10 mg but fell in females just below. It has been shown, e.g. (12) that the composition of the food markedly influences the utilization of food iron. As far as can be judged from the dietary interviews none of the 70-year-olds were vegetarians or otherwise kept to a diet that would significantly restrict the utilization of the food iron.

Potassium

Veiledning does not mention daily potassium intakes in relation to evaluation of diets. RDA 1974 just states that healthy adults need about 2.5 g per day of potassium (about 65 mmol). Judge and Cowan (34) claim that the minimal satisfactory dietary intake of potassium per day is 60 mmol. The average potassium intake in the present study 79 mmol in males and 68 mmol in females thus seems acceptable. However, probands in the lower quartile regarding potassium intake might be judged to have a low intake especially in females.

Vitamin A

Veiledning's 3-step-scale regarding vitamin A gives values of 750-600-400 µg retinol. The RDA 1974 value is 5000 IU (males) and 4000 IU (females) which for American dietary habits means 870 µg and 700 µg retinol for males and females respectively (22). The average retinol intake in the present study was thus very high. The lower decantile value was however lower.

16 males (8.8 per cent) and 23 females (12.2 per cent) had a protein intake of less than 0.7 g per kg body weight. The quality of the protein in the food was apparently good as the main part was derived from milk products, meat, fish and egg.

Fat

The average proportion of energy from fat was 38 per cent in both sexes. This is a higher value than the often recommended 25-35 per cent (e.g. Veiledning). However, it is not exceptionally high but in the same order of magnitude as in the Food Balance Sheet in Sweden at the time of the investigation (54): 0.8 per cent of male probands and 1.6 per cent of females showed a proportion of energy from fat of less than 25 per cent and 70.0 per cent (males) and 70.2 per cent (females) such a proportion of more than 35 per cent.

Calcium

As far as calcium is concerned, Veiledning considers 800 mg as a high intake, 600 mg as acceptable and 400 mg as a low/minimum intake. The corresponding RDA 1974 figure is 800 mg. Thus, the average intake of this study was high according to Veiledning and above the RDA value. The lower decentile values were not below the low/minimum value of Veiledning, but fell below the RDA 1974 value by almost 300 mg.

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Both Veiledning and RDA 1974 give a recommended iron value of 10 mg. On an average the intake of iron was thus high. The lower decedtile value was in males above 10 mg but fell in females just below. It has been shown, e.g. (12) that the composition of the food markedly influences the utilization of food iron. As far as can be judged from the dietary interviews none of the 70-year-olds were vegetarians or otherwise kept to a diet that would significantly restrict the utilization of the food iron.

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than the RDA value in both sexes but higher than the acceptable value of Veiledning in males and higher than the low/minimum value of Veiledning in females

Thiamin

Veiledning = proposed high intake of thiamin as well as the RDA 1974 recommended intake is 0.5 mg per 1000 kcal/4.2 MJ. On an average the intake fell above this recommended intake. The lower decantile values fell above the acceptable value of Veiledning (0.4 mg per 1000 kcal/4.2 MJ). Four males and two females fell below this value and only one proband (a male) fell below the low/minimum value of Veiledning (0.3 mg per 1000 kcal/4.2 MJ). RDA suggest however on the basis of the works by Horwitt et al (30) and Oldham (42) who propose that older persons use thiamin less efficiently a daily intake of 1 mg even with a daily energy intake of less than 2000 kcal/8.4 MJ. In the present material 15 males (8 per cent) and 50 females (27 per cent) showed a daily intake of less than 1 mg thiamin.

Riboflavin

Veiledning's high, acceptable and low/minimum values of riboflavin are 24 micrograms, 20 micrograms and 14 micrograms per kg body weight respectively. The value of RDA is 0.6 mg per 1000 kcal/4.2 MJ. Thus the average intake in this study corresponded to the high intake value of Veiledning and fell well above the RDA 1974 value. The lower decantile values however fell close to the low/minimum value of Veiledning and well below the RDA 1974 value. In fact 61 males (33.5 per cent) and 60 females (31.9 per cent) had an intake of less

than Veiledning = acceptable value (20 µg per kg body weight) and 20 males (11 % per cent) and 17 females (9 % per cent) below the low/minimum value of Veiledning (14 µg per kg body weight)

Ascorbic acid

The RDA 1974 value of ascorbic acid is 45 mg Veiledning gives the values corrected for losses during storage and preparation. The high value of Veiledning is 30 mg the acceptable value 20 mg and the low/minimum value is 10 mg. These figures are claimed to be comparable with the American figure (22). Intake of ascorbic acid was thus on average very high. The lower decedtile value was however somewhat below RDA 1974.

Only about 3 per cent of the 70-year-olds were found to have such advanced physical or mental handicaps that care in hospitals, nursing-homes or homes for the elderly was obviously indicated. Another 2 per cent of the population needed help with activities of daily living (8). Furthermore a certain number of probands were under treatment for diseases such as cardiac insufficiency and rheumatoid arthritis which obviously must have influenced their physical activity and the need of energy and nutrients.

Thirteen per cent of the 70-year-olds reported that their main mode of transportation within the city during the summer half of the year was walking or riding a bicycle. This group of individuals was found to have an average dietary energy intake of 2421 kcal/10.2 MJ per day (males) and 2070 kcal/8.7 MJ (females) compared with 2328 kcal/9.8 MJ and 1911 kcal/8.0 MJ respectively in the rest of the material indicating that this group of physically relatively active people might to a certain degree have been responsible for

the right part of the distribution curve of energy intake. These differences were however not statistically significant ($p > 0.05$ Student's t-test).

The meal pattern of the main part of the probands with at least one hot meal and one acceptable other meal per day seemed to be acceptable. Our results indicate that no male proband and only 1 per cent of the females did not have at least one hot meal a day. Furthermore in no probands from a nutritional aspect lesser good meals like coffee and buns seem to have been dominating the intake of energy and nutrients.

Thus the average 70-year-old person in Gothenburg had apparently adequate intake of food and good dietary habits. As will be reported elsewhere the body composition was found to be rather similar to that of previously investigated middle-aged individuals regarding body fat (50). The variations in intake of energy and nutrients were not greater than in an earlier investigated middle-aged group of females (10). Further detailed analyses of our results will aim at answering the question as to what extent the low intake values were shown by people with diseases in this age group.

Earlier studies of dietary habits and/or intake of energy and nutrients in Sweden include interview studies in elderly people living in homes for the elderly in Stockholm (52) in elderly non-institutionalized people and in pensioners living in homes for the elderly in Uppsala and surroundings (55) in a 75-year-old women in Gothenburg (27) in people in all Sweden - up to 75 years of age (33) in elderly people living alone and in households with others in Stockholm (43) and in all Sweden - up to 70 years of age (9). Söderberg (52), Hallberg (27) and Werner and Berfénstam (55) used the 24-hour recall method and calculated the intake of energy and nutrients. These three studies show lower values of intake of energy than the present

study The non-response group was as large as 43 per cent in the 75-year-old group in the study by Hallberg (27) In the studies by Johansson (33) and Arvidsson et al (9) emphasis was laid on description of meal habits and meal composition expressed in terms of food groups but data for the comparison of intake of energy and nutrients with the present study are not available

People with dentures in both jaws at the average age of 65 have been studied in the town of Umeå in northern Sweden with the 7 - day dietary record technique (53) The energy intake was on average lower (males 7.7 MJ females 6.3 MJ) than in the present study It is tempting to interpret the difference between their data and the present data as a difference dependent on differences in chewing ability in the two samples A comparison of energy intake in people with different dental state (evaluated by Tor Österberg DOS) in the present study showed however that the dental state influenced the choice of food but not significantly the intake of energy (29)

Elderly people in a rural district in southern Sweden (Dalby) have been studied with the duplicate portion technique (1 2 3 14 26 45) which has apparently given systematically lower values of intake of energy and nutrients than the present study - for a discussion of methodological differences between the duplicate portion technique and the dietary history method see (51) The constantly lower figures for intake of energy and nutrients with the duplicate portion technique might be due to difficulties for the probands to really maintain their ordinary food habits during the artificial experimental situation in which just as much food as that eaten is taken away for chemical analyses

Outside Scandinavia certain nutrition studies have been performed in industrial countries with a social

structure and a climate not too different from that of Sweden - for a review see (19) The comprehensive study performed in U K 1967-68 by the Panel on Nutrition of the Elderly (44) was mainly based on the 7-day record method Unfortunately that study suffered from a non-response rate of on average 44 per cent and in certain regions of as much as 68 per cent of the numbers drawn in the samples Certain comparisons of responders and non-responders and analyses of the representativity of the response group were performed In areas with a lower non-response rate (e g Rutherglen with a non-response rate of 19 per cent) the observed energy intake was in the same order of magnitude (2320 kcal/9.7 MJ) for males but seemed to be lower (1735 kcal/7.3 MJ) for females compared to the present study

In another nutrition survey in U K performed in 1969-1972 on 77 men and 187 women over the age of 65 and living at home (38 39 40) the mean intake of energy was 2300 kcal/9.7 MJ for men and 1750 kcal/7.3 MJ in women The non-response rate was however about 30 per cent and analyses of the representativity of the participants were not reported The limited possibilities of comparisons of nutritional data between only age based samples are illustrated by e g that height and weight of the 65-74 year-old males in that study were 166 ± 5 (M \pm SD) cm and 67 ± 14 kg which are approximately 7 cm shorter and 9 kg less than the 70-year-olds in Gothenburg The English women aged 65-74 years had a body weight of 64 ± 10 kg and a height of 152 ± 7 cm which means that they were of about the same body weight but approximately 8 cm shorter than the 70-year-old females in Gothenburg

In Gothenburg there are possibilities for comparisons of nutritional data between individuals in different ages in the same urban area These comparisons can only be made at present on a cross-sectional and not

on a longitudinal basis. A comparison between this study and studies on e.g. middle-aged women in Gothenburg shows that the energy intake (10) and the body composition (49) in females is in the same order of magnitude in these two groups as far as can be judged from cross-sectional studies.

This might mean that the physical activity of the 70-year old female is on an average similar to that of middle-aged females in Gothenburg. If the capacity of the elderly to utilize food is lower than in middle-aged women a change in the relationship between body fat and body cell mass should have been demonstrated. The body cell mass calculated from measurements of total body potassium does not seem to be lower in 70-year-old females than in middle-aged women (49). However, ageing per se in fact might cause a gradual decrease in body cell mass as proposed in earlier studies (7, 16, 25, 41, 48). The apparently same amount of body cell mass in females at the age of 70 and in middle life found in these cross-sectional studies might then be a result of selective mortality between these ages and/or secular factors.

Our conclusions concerning dietary habits in the 70-year-olds are thus in good agreement with the overall impression from the population study of 70-year-old people in Gothenburg, that this age group in general comprises individuals with rather good health.

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BODY COMPOSITION
IN 70-YEAR-OLD MALES AND FEMALES IN GOTHENBURG SWEDEN
A POPULATION STUDY

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ABSTRACT

The objectives of this substudy within the population study 70-year-old people in Gothenburg Sweden were to determine the body composition of the probands and to clarify possible relationships between results of these body compartment determinations and more simple somatometric variables. The cross-sectional data will be used as a basis for a prospective longitudinal geriatric/gerontological study. The subsample comprised 66 males and 76 females of whom 10 males

females underwent a complete examination. The probands examined were considered representative of the 70-year-old population in Gothenburg. Total body potassium was estimated by using a whole body counter and total body water with an isotope dilution technique using tritiated water as a tracer. Body cell mass, intracellular and extracellular water, body fat and fat-free extracellular solids were then calculated from body weight, total body potassium and total body water, using certain assumptions, the relevance of which in this age group is discussed. Average values of body weight were 76 kg and 66.3 kg, of body cell mass 28.1 kg and 20.3 kg, and of body fat 12.5 kg and 18.1 kg in males and females, respectively. It was possible from body weight and subscapular skinfold in males and from body weight, thigh and triceps skinfold in females to reach a proportion of explained variance of body fat of 58 per cent and 76 per cent, respectively. There was a good correlation between body fat and the difference between body weight and ideal body weight, as defined by Lindberg and collaborators (1956). A preliminary comparison between the current data and corresponding data from cross-sectional studies of middle-aged individuals in Gothenburg showed that the amount of fat was similar in the two age groups. Body cell mass seemed to be of the same order of magnitude in 70-year-old as in middle-aged females, but showed a lesser value in 70-year-old than in middle-aged males.

INTRODUCTION

The composition of the human body reflects genetic and environmental factors such as physical activity.

nutrition and certain diseases. To what extent the ageing processes per se influence the body composition is only known fragmentarily but there is reason to believe that the body cell mass will decrease at higher ages (1 5 11 20 23). Furthermore changes in cardiac and renal functions that might influence the amount of body water are common in higher ages even in individuals without obvious symptoms of cardiac or renal diseases.

There is reason to believe that the body composition is also of relevance to the level of different compounds in the blood such as creatinin, triglycerides and free fatty acids. A more detailed knowledge of the body composition is thus also of importance for a wider knowledge of the blood homeostasis of certain components especially the blood concentration of those used as an indication of the presence or absence of diseases. Furthermore the distribution of certain drugs depends to some extent on the relative size of the different body compartments that might influence drug tolerance.

Determination of body composition in higher ages has thus not only a general gerontological interest but also importance from many geriatric aspects.

The aim of the present study was to determine body composition in a Swedish population of 70-year-old people. The study is on the one hand a part of a broad gerontological/geriatric investigation of social and medical conditions of this age group (21) and on the other a part of a systematic survey of dietary habits and body composition in different age groups in Gothenburg (3 25 26). The objectives were also to clarify possible relationships between results of body compartment determinations with isotope techniques and more simple somatometric variables.

MATERIAL

The general design of the population study - the total sample comprised 1148 70-year-old persons (521 males and 627 females) - the sampling procedure analysis of the non-response and a broad presentation of investigation procedures have been presented earlier (21)

The subsample chosen for the body composition study was composed of those probands who underwent the examination programme at the hospital during the period February 14th to May 26th and September 11th to September 22nd 1972 as well as the dietary survey. This subsample comprised 142 probands (66 males and 76 females). 59 males and 62 females actually took part in the study which means a non-response rate of 11 per cent in males and 18 per cent in females. The causes of non-response are given in Table I. Furthermore in 10 males and 6 females the examination was considered incomplete or technically unsuccessful on the basis of the criteria reported later on.

Table I Causes of non-response

	Males	Females
Refusal	2	8
Severe illness	4	2
Institutional care outside Gothenburg	1	3
Claimed claustrophobia	0	1

Thus 49 males and 56 females were considered to have undergone a complete examination and the results are based on data on these probands

In order to test the representativity of the examined probands they were compared with other propositi in the study regarding sex marital status proportion of male probands registered in the Register of Temperance Board income and community rent allowances and with other probands in the study regarding height body weight waist girth triceps skinfold and subscapular skinfold (Table II) There were no significant differences on the one per cent level (χ^2 -test and Student's t-test respectively) in these respects between the examined group and other propositi and probands respectively

METHODS

Body composition data were calculated from measurements of body weight (BW) total body potassium (TBK) and total body water (TBW)

Total body potassium was estimated by using a high sensitivity whole body counter utilizing plastic scintillators (24) to detect the gamma radiation from the naturally occurring radionuclide ^{40}K which is a constant fraction of all natural potassium. The counter had been calibrated by the administration of ^{42}K to volunteers. This calibration is described elsewhere (2). The total expected standard deviation of a single potassium determination is approximately 80 mmol including random errors due to

Table II Average values of height body weight waist girth triceps and subscapular skinfold in those probands in whom body composition was examined and in other probands (Student's t-test NS: $p > 0.05$)

	Males			Females		
	Examined	Not examined	Sign	Examined	Not examined	Sign
	n=49	n=402		n=56	n=466	
Height (cm)	173	174	NS	160	160	NS
Body weight (kg)	76.2	76.2	NS	66.3	66.8	NS
Waist girth (cm)	90.4	89.7	NS	82.3	83.3	NS
Triceps skinfold (mm)	9.2	8.6	NS	20.0	18.1	NS
Subscapular skinfold (mm)	15.4	14.3	NS	17.5	18.0	$p < 0.05$
						NS

the counting statistics calibration uncertainties and uncontrollable variations in the measuring procedures. The subjects were asked to bath and wash their hair the day before the measurement during which they were dressed in underwear and a hospital gown previously checked to contain no radioactive contamination. The count duration for each subject was 100 seconds.

Total body water was determined with an isotope dilution technique using tritiated water (THO) as a tracer (5-17). 400 μ Ci THO was given perorally and was equilibrated for five hours. The specific activity (THO/ H_2O) in urine was calculated from measurements of two consecutive voidings two hours apart collected after the bladder had been emptied at the end of the equilibrium period. The urine samples were distilled on a sand-bath. Of the colour-free distillate 0.5 ml was mixed in duplicate with 10 ml of a scintillation liquid (200 g Naphtalene, 25 g PPO, 100 mg POPOP in 2000 ml Dioxane) and measured in a liquid scintillation counter (Tri-Carb, Packard Instruments Co.). Such determinations were rejected where only one urine sample was available or where the difference in specific activity between the two samples exceeded 10%.

Calculations. Most of the body potassium (about 98 per cent) is located intracellularly and was during the calculations considered to be in constant relation with the cell mass. The water content of the body cells was also considered to be constant. It was thus possible to estimate body compartments from determinations of BW, TBW and TBW.

Body cell mass (BCM) in kg was calculated as $TBW \text{ (in mmol)} \times 8.33 \times 10^{-3}$ (19). Intracellular water (ICW) in kg was calculated as 78 per cent of BCM. Extracellular water (ECW) in kg was calculated as $TBW - ICW$. Body fat (BF) in kg was calculated as $BW - (BCM + ECW + FFECS)$ where FFECS (fat-free extracellular solids) were approximated at 12 per cent of BW (5-17).

Table II Average values of weight body weight waist girth triceps and subscapular skinfold in those probands in whom body composition was examined and in other probands (Student's t-test NS: $p > 0.05$)

	Males			Females		
	Examined	Not examined	Sign	Examined	Not examined	Sign
	n=49	n=402		n=56	n=466	
Height (cm)	173	174				
Body weight (kg)	76.2	76.2	NS	160	160	NS
Waist girth (cm)	90.4	89.7	NS	66.3	66.8	NS
Triceps skinfold (mm)	9.2	8.6	NS	82.3	83.3	NS
Subscapular skinfold (mm)	15.4	14.3	NS	20.0	18.2	$p < 0.05$
				17.5	18.0	NS

- subject standing The mean difference between the two observers was -4.0 ± 0.5 mm (M \pm SE)

None of the above mentioned differences differed significantly ($p > 0.05$ Student's t-test) from zero

When studying correlations to body fat the difference between BW and ideal body weight was used. The ideal body weight in kg was calculated for males as (height in cm $\times 0.71$) - 53.9 and for females as (height in cm $\times 0.67$) - 48.1 from the data of Lindberg et al (16)

R e l a t i o n a n a l y s e s In order to analyze the possible relation between the results and certain characteristics of the probands the following dicotomic contrast groups were studied regarding the body composition data:

- I Diuretic treatment Probands with (6 males and 18 females) contra probands without (43 males and 38 females) diuretic treatment (thiazides, other diuretic sulfonamide compounds or ethacrynic acid)
- II Congestive heart failure Probands with (14 males and 7 females) contra probands without (35 males and 49 females) congestive heart failure (two of the symptoms oedema, cyanosis and dyspnoea at the general medical examination and a calculated heart volume of 500 ml per m² body surface or more (males) and 450 ml per m² body surface (females) respectively and/or three of those symptoms and/or digitalis treatment and heart volume as above and/or heart volume as above in absence of hypertension (see below) and/or congestion of the pulmonary vessels judged by the roentgenologist)
- III Hypertension Probands with (7 males and 20 females) contra probands without (42 males and 36 females) hypertension (treatment with anti-hypertensive drugs and/or a diastolic blood pressure of 115 mm Hg or more and/or anamnestic hypertension and a heart volume as above in absence of congestive heart failure (see II))

S k i n f o l d measurements were performed with a Harpenden caliper (10) with a pressure of 10 g/mm^2 applied at a contact surface of 20 mm^2 . The skinfold was measured 1) at the back of the right upper arm midway between acromion and elbow with the arm hanging relaxed and with the fold running parallel to the length of the arm - subject standing - (triceps skinfold) 2) just below the angle of the right scapula with the fold parallel to the natural cleavage line of the skin - subject standing - (subscapular skinfold) 3) just above the right anterior superior iliac spine with the fold parallel to the natural cleavage line of the skin - subject in supine position - (suprailiac skinfold) and 4) at the front of the right thigh midway between groin and patellar base with the fold vertical - subject standing with slight flexion in the hip and knee joints (thigh skinfold). Triceps and subscapular skinfolds were measured on all probands by one and the same registered nurse who was specially trained for the purpose. Three measurements were taken at each position and the results were based on the average value of these observations. Suprailiac and thigh skinfolds were measured - one observation per measured proband - in a subsample ($n=329$) including those probands who underwent the body composition study by two observers (one of us /T L / and a specially trained technician). An inter-observer variation study in these two observers revealed a mean difference of $-0.2 \pm 0.3 \text{ mm}$ ($M \pm SE$) and $-0.5 \pm 0.5 \text{ mm}$ for suprailiac and thigh skinfolds respectively.

U p p e r a r m g i r t h was measured over the midpoint of head of the right biceps muscle - subject standing with upper arm slightly abducted and elbow flexed at 90° . The mean difference between the two observers was $-1.0 \pm 1.0 \text{ mm}$ ($M \pm SE$).

W a i s t g i r t h was measured horizontally halfway between the lowest floating ribs and iliac crests

- subject standing The mean difference between the two observers was -4.0 ± 0.5 mm (M \pm SE)

None of the above mentioned differences differed significantly ($p > 0.05$ Student's t-test) from zero

When studying correlations to body fat the difference between BW and ideal body weight was used. The ideal body weight in kg was calculated for males as (height in cm \times 71) - 53.9 and for females as (height in cm \times 67) - 48.1 from the data of Lindberg et al (16)

Relation analyses In order to analyze the possible relation between the results and certain characteristics of the probands the following dicotomic contrast groups were studied regarding the body composition data

- I Diuretic treatment Probands with (6 males and 18 females) contra probands without (43 males and 38 females) diuretic treatment (thiazides, other diuretic sulfonamide compounds or ethacrynic acid)
- II Congestive heart failure Probands with (14 male and 7 females) contra probands without (35 males and 49 females) congestive heart failure (two of the symptoms: edema, cyanosis and dyspnoea at the general medical examination and a calculated heart volume of 500 ml per m² body surface or more (males) and 450 ml per m² body surface (females) respectively and/or three of those symptoms and/or digitalis treatment and heart volume as above and/or heart volume as above in absence of hypertension (see below) and/or congestion of the pulmonary vessels judged by the roentgenologist)
- III Hypertension Probands with (7 males and 20 females) contra probands without (42 males and 36 females) hypertension (treatment with anti-hypertensive drugs and/or a diastolic blood pressure of 115 mm Hg or more and/or anamnestic hypertension and a heart volume as above in absence of congestive heart failure (see II))

Skinfold measurements were performed with a Harpenden caliper (10) with a pressure of 10 g/mm^2 applied at a contact surface of 20 mm^2 . The skinfold was measured 1) at the back of the right upper arm midway between acromion and elbow with the arm hanging relaxed and with the fold running parallel to the length of the arm - subject standing - (triceps skinfold) 2) just below the angle of the right scapula with the fold parallel to the natural cleavage line of the skin - subject standing - (subscapular skinfold) 3) just above the right anterior superior iliac spine with the fold parallel to the natural cleavage line of the skin - subject in supine position - (suprailiac skinfold) and 4) at the front of the right thigh midway between groin and patellar base with the fold vertical - subject standing with slight flexion in the hip and knee joints (thigh skinfold). Triceps and subscapular skinfolds were measured on all probands by one and the same registered nurse who was specially trained for the purpose. Three measurements were taken at each position and the results were based on the average value of these observations. Suprailiac and thigh skinfolds were measured - one observation per measured proband - in a subsample ($n=329$) including those probands who underwent the body composition study by two observers (one of us /T L / and a specially trained technician). An inter-observer variation study in these two observers revealed a mean difference of $-0.2 \pm 0.3 \text{ mm}$ ($M \pm SE$) and $-0.5 \pm 0.5 \text{ mm}$ for suprailiac and thigh skinfolds respectively.

Upper arm girth was measured over the midpoint of head of the right biceps muscle - subject standing with upper arm slightly abducted and elbow flexed at 90° . The mean difference between the two observers was $-1.0 \pm 1.0 \text{ mm}$ ($M \pm SE$).

Waist girth was measured horizontally half-between the lowest floating ribs and iliac crests

subject standing The mean difference between the two observers was -4.0 ± 0.5 mm (M \pm SE)

None of the above mentioned differences differed significantly ($p > 0.05$ Student's t-test) from zero

When studying correlations to body fat the difference between BW and ideal body weight was used The ideal body weight in kg was calculated for males as (height in cm $\times 0.71$) -53.9 and for females as (height in cm $\times 0.67$) -48.1 from the data of Lindberg et al (16)

Relation analyses In order to analyze the possible relation between the results and certain characteristics of the probands the following dicotomic contrast groups were studied regarding the body composition data:

- I Diuretic treatment Probands with (6 males and 18 females) contra probands without (43 males and 38 females) diuretic treatment (thiazides other diuretic sulfonamide compounds or ethacrynic acid)
- II Congestive heart failure Probands with (14 males and 7 females) contra probands without (35 males and 49 females) congestive heart failure (two of the symptoms oedema cyanosis and dyspnoea at the general medical examination and a calculated heart volume of 300 ml per m² body surface or more (males) and 450 ml per m² body surface (females) respectively and/or three of those symptoms and/or digitalis treatment and heart volume as above and/or heart volume as above in absence of hypertension (see below) and/or congestion of the pulmonary vessels judged by the roentgenologist)
- III Hypertension Probands with (7 males and 20 female) contra probands without (42 males and 36 females) hypertension (treatment with anti-hypertensive drugs and/or a diastolic blood pressure of 115 mm Hg or more and/or anamnestic hypertension and a heart volume as above in absence of congestive heart failure (see II))

- IV Prostatic enlargement and/or prostatism Male probands with (n=15) contra male probands without (n=34) prostatic enlargement judged by the physician at the general medical examination and/or symptoms of prostatism based on clinical evaluation of anamnestic data
- V Urinary infection Female probands with (n=6) contra females without (n=50) urinary infection (positive or doubtful result from first Cultube® test - for bacteriuria - and positive result from a second Cultube® test)

RESULTS

Waist girth triceps skinfold and subscapular skinfold are given in Table II. In the body composition examined subsample upper arm girth was 294 ± 22.3 mm (M \pm SD) and 299 ± 36.7 mm suprailiac skinfold 13.9 ± 6.61 mm and 19.9 ± 8.77 mm and thigh skinfold 17.0 ± 10.2 mm and 27.9 ± 11.0 mm in males and females respectively.

Height BW TBW and TBK are given in Table III. TBW constituted on an average 63 per cent of BW in males and 54 per cent in females. The different body composition data is calculated both in absolute and relative terms (related to BW) in Table IV. The mean ratio between ECW and ICW was 1.21 in males and 1.27 in females. Average BCM and ECW were both more than twice as great as average BF in males whilst in females all these three compartments were of the same order of magnitude.

The correlation coefficients between BW BCM ECW and BF versus different anthropometric measurements are

Table III. Average values of height, body weight (BW), total body water (TBW) and total body potassium (TBK). SD: standard deviation

	Males (n=49)		Females (n=56)	
	Mean	SD	Mean	SD
Height (cm)	173	5.9	160	6.0
BW (kg)	76.2	11.1	66.3	12.4
TBW (kg)	48.1	6.5	35.8	5.5
TBK (mmol)	3360	422	2433	323

Table IV. Average values of body cell mass (BCM), extra-cellular water (ECW) and body fat (BF) in absolute values and in per cent of body weight (BW). SD: standard deviation

	Males		Females	
	Mean	SD	Mean	SD
Absolute values (kg)				
BCM	28.1	3.5	20.3	2.7
ECW	26.5	5.0	20.0	4.3
BF	12.5	6.5	18.1	7.2
Relative values (per cent of BW)				
BCM	37.1	4.0	30.9	4.0
ECW	34.9	5.6	30.2	4.9
BF	16.1	7.0	26.6	6.7

- IV Prostatic enlargement and/or prostatism Male probands with (n=15) contra male probands without (n=34) prostatic enlargement judged by the physician at the general medical examination and/or symptoms of prostatism based on clinical evaluation of anamnestic data
- V Urinary infection Female probands with (n=6) contra females without (n=50) urinary infection (positive or doubtful result from first Cultube® test - for bacteriuria - and positive result from a second Cultube® test)

RESULTS

Waist girth triceps skinfold and subscapular skinfold are given in Table II In the body composition examined subsample upper arm girth was 294 ± 22.3 mm (M \pm SD) and 299 ± 36.7 mm suprailiac skinfold 13.9 ± 6.6 mm and 19.9 ± 8.7 mm and thigh skinfold 17.0 ± 10.2 mm and 27.9 ± 11.0 mm in males and females respectively

Height BW, TBW and TBK are given in Table III TBW constituted on an average 63 per cent of BW in males and 54 per cent in females The different body composition data is calculated both in absolute and relative terms (related to BW) in Table IV The mean ratio between ECW and ICW was 1.21 in males and 1.27 in females Average BCM and ECW were both more than twice as great as average BF in males whilst in females all these three compartments were of the same order of magnitude

The correlation coefficients between BW BCM ECW and BF versus different anthropometric measurements are given in Tables V-VI

Table VI Correlation coefficients between body weight (BW) body cell mass (BCM) extracellular water (ECW) body fat (BF) and other variables females (S: significance from zero xxx $p < 0.001$ xx $p < 0.01$ x: $p < 0.05$ NS: $p > 0.05$)

	Females									
	BW		BCM		ECW		BF			
	r	S	r	S	r	S	r	S	r	S
Height	0.40	xx	0.35	xx	0.47	xxx	0.19	NS		
Body weight	-		0.69	xxx	0.66	xxx	0.83	xxx		
Body cell mass	0.69	xxx	-		0.38	xx	0.44	xxx		
Extracellular water	0.66	xxx	0.38	xx	-		0.26	x		
Body fat	0.83	xxx	0.44	xxx	0.26	x	-			
Upper arm girth	0.86	xxx	0.64	xxx	0.66	xxx	0.67	xxx		
Waist girth	0.85	xxx	0.52	xxx	0.57	xxx	0.75	xxx		
Triceps skinfold	0.69	xxx	0.48	xxx	0.51	xxx	0.56	xxx		
Subscapular skinfold	0.67	xxx	0.51	xxx	0.26	x	0.67	xxx		
Suprailiac skinfold	0.64	xxx	0.42	xx	0.38	xx	0.59	xxx		
Thigh skinfold	0.50	xxx	0.30	x	0.10	NS	0.57	xxx		

Table V Correlation coefficients between body weight (BW) body cell mass (BCM) extracellular water (ECW) body fat (BF) and other variables males (S: significance from zero xxx: $p < 0.001$ xx: $p < 0.01$ x: $p < 0.05$ NS: $p > 0.05$)

Males									
	BW		BCM		ECW		BF		
	r	S	r	S	r	S	r	S	
Height	0.61	xxx	0.63	xxx	0.41	xx	0.27	NS	
BW	-	-	0.58	xxx	0.60	xxx	0.69	xxx	
BCM	0.68	xxx	-	-	0.41	xx	0.16	NS	
ECW	0.60	xxx	0.41	xx	-	-	-0.09	NS	
BF	0.69	xxx	0.16	NS	-0.09	NS	-	-	
Upper arm girth	0.74	xxx	0.51	xxx	0.40	xx	0.53	xxx	
Waist girth	0.84	xxx	0.45	xxx	0.47	xxx	0.66	xxx	
Triceps skinfold	0.46	xxx	0.05	NS	0.27	NS	0.47	xxx	
Subscapular skinfold	0.56	xxx	0.04	NS	0.23	NS	0.65	xxx	
Suprailiac skinfold	0.64	xxx	0.23	NS	0.34	xx	0.58	xxx	
Thigh skinfold	0.58	xxx	0.31	x	0.34	xx	0.44	xx	

dependent variable: body fat independent variable: subscapular skinfold (SubST)

multiple regression analysis of the data from the 1970-1971 season. The results are shown in Table 1. The regression equation for the 1970-1971 season is:

[illegible]

All parameters showed highly significant correlations to BW. The highest figures ($r > 0.80$) were obtained for waist girth in males and upper arm girth waist girth and BF in females.

Fewer parameters showed high correlations to BCM. The highest figures ($r > 0.60$) were obtained for - apart from BW - height in males and upper arm girth in females.

The correlations to ECW were also weaker than to BW. Correlation coefficients higher than $r = 0.60$ were - apart from BW in females - only obtained for upper arm girth in females.

The correlations to BF were higher in females than in males regarding BW waist girth upper arm girth thigh skinfold, triceps skinfold and BCM. Regarding subscapular and suprailiac skinfolds the correlations were of the same order of magnitude in both sexes.

The correlation coefficient between BF and the difference between BW and ideal body weight (16) was $r = 0.71$ in males and $r = 0.91$ in females. The regression equation was $BF = 0.46(BW - \text{ideal body weight}) + 9.4$ in males and $BF = 0.54(BW - \text{ideal body weight}) + 14.5$ in females.

The result of a multiple regression analysis with BF as the dependent variable is given in Table VII. For both sexes BW was the most important independent variable with a R-value of 0.69 in males and 0.83 in females. In males R increased to 0.76 when subscapular skinfold (SubSF) was also taken into consideration. In females the R-value was 0.87 if thigh skinfold (ThSF) and triceps skinfold (TSF) were also taken into account. The regression equation after two analysis steps in males was $BF \text{ (in kg)} = 0.28BW \text{ (in kg)} + 0.51 \text{ SubSF (in mm)} - 16.3$. The corresponding equation after

three analysis steps in females was BF (in kg) = 0.49
 BW (in kg) + 0.16 ThSF (in mm) - 0.30TSF (in mm) - 13.6

With the analysis of possible relations between the body composition data and other data concerning these 70-year-olds namely the presence of diuretic treatment congestive heart failure hypertension prostatic enlargement/prostatism and urinary infection a low-grade significant difference was obtained with only one comparison; congestive heart failure in females. Regarding TBW slightly higher values in both sexes were obtained in congestive heart failure but the difference was thus only statistically significant ($p < 0.05$ Student's t-test) in females (Table VIII).

Table VIII Average body weight (BW) total body water (TBW) and total body potassium (TBK) in probands with cardiac decompensation (A) and in other probands (B) (Student's t-test NS: $p > 0.05$)

	Males			Females		
	A (n=14)	B (n=35)	Sign	A (n=7)	B (n=49)	Sign
BW	78.8	75.1	NS	73.9	65.2	NS
TBW	50.6	47.5	NS	40.1	35.2	$p < 0.05$
TBK	3410	3352	NS	2569	2415	NS

DISCUSSION

As in the tests of representativity of the examined group no significant differences at the one per cent level were obtained between the examined subsample and other propositi and probands respectively the examined group seemed to be representative for the total material. As shown earlier in detail (21) the total material was also generally representative of 70-year-old people in Gothenburg.

Total body water and total body potassium have been previously studied in higher age groups. Calculations of body fat on the assumptions used in this study have only been performed previously in studies of younger age groups. One may question whether the assumptions which the calculations of the body composition were based on are correct in the present age group.

One possibility may be that the amount of fat-free extracellular solids has decreased due to the ageing of the bone mass. Durnin and Womersley (9) have compiled data from different investigations using a variety of experimental techniques. They found the amounts of skeletal bone reported to be 8 to 15 per cent lower in 75-year-old than in 50-year-old males and about 18 per cent to 30 per cent lower in 75-year-old than in 45-year-old females.

If the average loss of bone from middle age is roughly estimated to be 10 per cent in males and 25 per cent in females this would make a small but significant change in the overall gross weight of the skeleton. With the calculation of body fat this would cause a false low value. On the other hand it is possible that the amount of extracellular connective tissue

increases at higher ages which means that the proportion of fat-free extracellular solids might be augmented in higher age groups. To what extent this might with the calculations compensate for the above-mentioned loss of fat-free extracellular solids in bone tissue is hard to predict. The fact that more than 3/4 of FFECS are present in the skeleton (19) indicates however that small variations in collagen concentration and collagen amount in other organs in the body should not markedly influence the results. If no such compensation existed the error of the present estimation of BF should however not exceed an average order of magnitude of 1 kg in males with a body weight of 76 kg and 2 kg in females with a body weight of 66 kg. Thus on average the method might apparently underestimate the amount of body fat in males by about 8 per cent of 12.5 kg and in females by about 10 per cent of 18 kg.

Another possibility might be that the factors used for the calculations of body cell mass and intracellular water, i.e. 120 mmol potassium and 780 g water per kg cell mass, are not valid for aged subjects. The estimation of these factors is in general based on data on healthy young or middle-aged individuals. Older rats have been shown to have a lower intracellular potassium concentration (12-14) than younger animals. However, available data on healthy subjects supports the opinion that the factors are just as valid at higher ages as in younger ones (6, 19, 28). Furthermore, a similar intracellular potassium concentration has been found (7) in old patients with senile dementia.

Intracellular potassium concentration is decreased in some clinical disorders and medical treatment, i.e. starvation (15). None of the participants in the present material suffered from such conditions. However, not all probands were healthy. 21 probands were judged to have congestive heart failure and 27 probands to

have hypertension conditions which might be suspected to be related to changes in the amount of TBW. Furthermore diuretic treatment present in 11 of these males and 18 of these females might influence total body potassium and body water. Only congestive heart failure in females was however found to have a slight influence on the variables studied with an increased TBW.

Fifteen of the males in the present material were judged to have prostatic enlargement and/or prostatism and 6 females to have urinary infection conditions which might be suspected to cause some residual urine. This might influence the results since bladder urine does not equilibrate with body water as fast as other compartments do regarding tritiated water (5). Urine samples taken for analysis might thus have been diluted with residual urine with a lower specific activity. These persons were however included in the material since the difference in specific activity between the two urine portions did not exceed 10 per cent. In these persons no influence on the body composition data was observed with the statistical analysis of the differences between these probands and the rest of the examined probands. It should however be noticed that 3 of the 4 female probands whose TBW determinations were rejected due to a difference in specific activity between the two urine samples exceeding 10 per cent suffered from conditions that might be combined with an incomplete emptying of the bladder at the end of the equilibrium period (one female had been treated with radiation for cancer of the uterus, another had had an operation for a prolapse of the uterus and the third had a urinary infection). The possible limitation of the methods used for analysis of body composition in individuals in whom a residual urine is present should be taken into consideration. In those 10 males in whom the determination was rejected the production of only one urine sample was the reason for rejection in all cases but 3, in whom residual urine was also

increases at higher ages which means that the proportion of fat-free extracellular solids might be augmented in higher age groups. To what extent this might with the calculations compensate for the above-mentioned loss of fat-free extracellular solids in bone tissue is hard to predict. The fact that more than $3/4$ of FFECS are present in the skeleton (19) indicates however that small variations in collagen concentration and collagen amount in other organs in the body should not markedly influence the results. If no such compensation existed the error of the present estimation of BF should however not exceed an average order of magnitude of 1 kg in males with a body weight of 76 kg and 2 kg in females with a body weight of 66 kg. Thus on average the method might apparently underestimate the amount of body fat in males by about 11 per cent of 12.5 kg and in females by about 10 per cent of 18 kg.

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Table IX Total body potassium per kg body weight from different studies Reference 1 21 24 present study; mean values Reference 8: median values

Reference	Age (ys)	Males		Females	
		Number	mmol K/ kg BW	Number	mmol K/ kg BW
1	58-68	30	45 1 ^a		
	60-67			18	32 6 ^a
	70-85	4	43 1 ^a		
	78-79			3	34 2 ^a
8	65-70	185	43 7 ^b	144	32 8 ^b
	70-75	97	43 1 ^b	44	31 6 ^b
21	55-65	42	47 8	54	38 2
	65-85	18	43 4	13	37 6
24	60-65	21	40 7 ^c		
	60-70			5	31 5 ^c
present study	70	49	44 1	56	36 7

a) calculated from values given for BW and total potassium

b) calculated from values given for BW and cell mass
Potassium concentration stated to be 92.5 mmol/kg cell mass

c) calculated from values given in diagrams

urine samples was found

The whole body counter has been used for estimating the total body potassium in different populations and cross-sectional data has been presented from e.g. Los Alamos New Mexico (1) Rochester Minnesota (20), Brookhaven New York (23) and Germany (8). As seen from Table IX the data from these studies and our studies (expressed as mmol TBK per kg BW) generally agree well. The only exception is the data from Brookhaven which is slightly lower than ours and the data from the other centres mentioned.

Some cross-sectional data on the total body water of the aged human has been published (13, 19). Our figures for the relative amount of total body water are higher than those from U.K. (13) and U.S.A. (19). The explanation may be that individuals in the material from U.K. and U.S.A. were generally somewhat fatter than their counterparts in Gothenburg. That Swedish people may have a lower amount of body fat than people in U.K. and U.S.A. is supported by comparisons of BW/height measurements and skinfold measurements in studies from these two countries (9, 18, 22) and the present material.

Differences in the results of body composition analyses between the studies mentioned and the present study might also at least to some extent be explained by different epidemiological sampling techniques. To what extent the above mentioned studies were performed as representative samples of the population is not clear.

Further observations that came out of this study were that males still at the age of 70 years had a higher relative amount of body cell mass than females and a slightly higher amount of extracellular water whilst their amount of body fat was almost only half of that

its relation to body fat. This ideal weight was based mainly on measurements on young and middle-aged individuals and the age range covered individuals up to 69 years. That material was not representative of the total population as it only covered individuals with working capacity and employed in a g different industries. As far as we know the present study is the first that concerns a representative sample of a higher age population. There was a rather good correlation between body fat and the difference between body weight and the ideal weight. Regression equations with body fat as the dependent and body weight and ideal body weight as independent variables were obtained which might be used in practical clinical work.

Epidemiological body composition studies have also been performed on younger populations in Gothenburg. A detailed comparison between the present data and data from the other studies cannot yet be performed as the complete basal data from these studies has not been published as yet. However, a preliminary comparison (25) with 54-year-old males (27) and females (4) shows that the amount of body fat was roughly the same in these two age groups. Body cell mass was lower in 70-year old males than in 54-year-old males but similar in females. One possible explanation might be that retired males at the age of 70 have a lower physical activity than an active middle-life and therefore have lower muscle mass. The same amount of body fat in the two male age groups might be a result of a decreased intake of energy. In females the data might be consistent with the hypothesis that both physical activity and energy intake are of the same order of magnitude in the two age groups.

However, comparisons between cross-sectional studies must be interpreted with great caution. An apparent change of a variable between two age groups might be

of the females. Contrary to younger people the ECW/ICW ratio was the same in both sexes in the present material and well above 1

It is hard to explain the sex difference regarding the correlation between BCM and BF ($r=0.16$ and $r=0.44$ for males and females respectively). Obviously the obese male might have a less developed muscle mass than the obese female. Still harder to explain is the relatively low correlation between BCM and height in females ($r=0.35$).

The determination of the body composition by whole-body counting and isotope dilution is a simple and reliable method and with a minimum of inconvenience to the patient. It has one drawback, however. The whole-body counter is only available at a few centres. One major aim of the present study therefore was to test whether any calculations based on anthropometric data could be used in order to estimate body fat and thus replace the present method. It was possible from body weight and subscapular skinfolds in males and from body weight, thigh and triceps skinfold in females to reach a proportion of explained variance of body fat of 58 per cent and 76 per cent respectively.

The higher proportion of explained variance and the higher correlation coefficients in females can be attributed to the higher amount of body fat in this sex. It also seems from this analysis that skinfold measurements on the trunk (subscapular skinfold) seem to be relatively more relevant for estimation of body fat in males than in females, whereas extremity skinfold measurements (triceps and thigh skinfold) seem to be more relevant in females than in males.

In this study the difference between the subjects' body weight and a standard ideal weight (16) widespread in Scandinavia and in much use in the clinics

at the estimation of overweight was tested regarding its relation to body fat. This ideal weight was based mainly on measurements on young and middle-aged individuals and the age range covered individuals up to 69 years. That material was not representative of the total population as it only covered individuals with working capacity and employed in e.g. different industries. As far as we know the present study is the first that concerns a representative sample of a higher age population. There was a rather good correlation between body fat and the difference between body weight and the ideal weight. Regression equations with body fat as the dependent and body weight and ideal body weight as independent variables were obtained which might be used in practical clinical work.

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However, comparisons between cross-sectional studies must be interpreted with great caution. An apparent change of a variable between two age groups might be

the result of - apart from changes of age per se - also secular changes and selective mortality

This paper deals with a part of a broad epidemiological study comprising anamnestic data about previous occupation living conditions physical activity etc and a detailed survey of present social and medical conditions A detailed analysis of the body composition data in relation to the other parameters must wait until a more thorough penetration and evaluation of this latter data has been performed

Furthermore the question whether e.g. tolerance of drugs is related to changes in body composition will be the subject of further studies

In the population of 70-year-olds in Gothenburg diseases with serious handicaps were unusual. However medical findings associated with disease were common. It is of great interest to try to define a group of probands without symptoms and signs of overt disease who roughly could be looked upon as healthy and use this group for comparisons with younger populations where disease is more uncommon. A separate comparison of the body composition of these healthy 70-year-olds with younger healthy groups is of course of great interest but must wait until other parts of the extensive material have been penetrated further

The study 70-year-old people in Gothenburg is designed to be a longitudinal prospective study and the probands will be reexamined at the age of 75

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Supplementum 612

The Interactions of SMOKING, ENVIRONMENT AND HEREDITY and Their Implications for Disease Etiology

*A Report of
Epidemiological Studies on the Swedish Twin Registries*

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Acta Medica Scandinavica

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ment of Medicine of the Karolinska Institute at the Serafimer
Hospital

The Interactions of

S M O K I N G E N V I R O N M E N T A N D H E R E D I T Y

and Their Implications for Disease Etiology

A Report of Epidemiological Studies on the Swedish Twin
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As long ago as the late fifties based on paper by Doll and Hill (1956) Hammond and Horn (1958) Dorn (1958 1959) and others great attention began to be paid to an association between tobacco smoking and disease above all lung cancer. The epidemiological evidence of causal connection was however questioned by several authors among whom Fisher (1958) and Berkson (1959) argued that the statistical relationship was not causal but dependent on factors related to the smoking habit as well as to disease. This discussion later became known as the cancer controversy an expression which was taken from a publication by Fisher in 1959 advancing the theory that smokers and nonsmokers are genetically different. The propensity to become smoker or at least to try smoker after having begun should in some way be genetically linked to an increased susceptibility to develop disease. During the years this theory has met strong opposition. It seems now to be ruled out by a majority of researchers in regard to certain respiratory diseases including lung cancer while few still question the causality (Korch 1973; Starling 1975). The genetic theory should however deserve serious attention in regard to other diseases that have been shown to be more prevalent among smokers than among nonsmokers - such as coronary heart disease an entity that in regard to numbers is far more important cause of death than is lung cancer.

The opinion in regard to smoking and health has been reflected in periodically appearing documents from the US Public Health Service such as the Surgeon General Reports (1964-1975) in reports from the Royal College of Physicians of London (1962 1971) and recently in a report from the World Health Organization (1975). The last mentioned report states that smoking is an important causative factor in lung cancer chronic bronchitis and emphysema ischemic heart disease and obstructive peripheral vascular disease. No recognition was given to the possibility that some of the apparent effects could be due to confounding of environmentally or genetically determined associations between smoking and the potentially hazardous habitual factors such as alcohol or drug abuse.

Whether completely environmentally determined or to some extent influenced by genetics as well it is obvious that smoking is more common in certain population groups defined by sex age area urban-rural residence education and occupation. There also are great differences between different countries. Further within individual the smoking habit is associated with drinking and food habits physical and social activities etc factors that may involve risk of their own for provoking disease. This may lend support to an assumption of smoker personality characterized by an outgoing life-style and tendency to neglect health affecting factors.

It is common knowledge that many disease entities to some extent are influenced by genetic factors. This influence does not have to be looked upon as causative \equiv 11 but may still play significant part in the late development of the disease. There is strong evidence for example that longevity is markedly influenced by the genetic code.

These considerations necessarily give support to the view that a scientific assessment of the association between smoking and disease experience could with few exceptions not be valid without control for possible intervening and confounding variables among which genetic factors may be of decisive importance. To meet this end a model should be developed to control for the influence of environmental covariables and genetic factors - making it to some extent possible to distinguish between the causal and constitutional hypotheses.

Against this background the Department of Environmental Hygiene of the Karolinska Institute in 1959 drew up plans for a continuing epidemiological research program based on population studies of twins.

A twin registry was compiled in 1959-1961 that covers about 11 000 same-sexed twin pairs born 1886-1923 with both partners alive at the time of compilation. A series of mailed questionnaires furnished information about smoking and drinking habits, a variety of dose-related variables and health parameters. Subsamples of the twins have subsequently been clinically examined to meet various specific purposes such as validation of questionnaire data. The twins have been followed up to obtain mortality data. The program was extended in 1967 when the same type of study was performed in collaboration with the Follow-up Agency of the National Research Council, National Academy of Sciences, Washington, DC. This study covered more than 4 000 male twin veteran pairs born 1917-1927 and belonging to the National Research Council (NRC) Twin Registry. A further expansion of the twin-program was afforded in 1971-1975 when the Swedish Twin Registry extended its files to include another approximately 13 000 same-sexed pairs born in 1926-1958 and alive in 1971.

Special methods have been developed to analyze the data, one main feature of which is to compare an evaluation of smoking relationships among all twins regarded as a sample of nonrelated individuals with results obtained in groups of smoking discordant twins. The methodology as well as the results of these studies have been reported in about 30 publications among which six are doctoral dissertations. Data on certain aspects are still not conclusive due to small numbers among other things; even so they do elucidate the smoking and health issue in a perspective that has hitherto been but little recognized. It has therefore been considered useful now to publish a comprehensive review of the research program and to present an overall assessment of the results.

Though one major aim is to review results reported in previous papers dealing with the registry, the present publication does contain a large amount of unpublished data, especially in the chapter dealing with the smoking related disease panorama. The latest report on mortality appeared in 1973 and covered an 11-year follow-up including June 1972. Data from another three years of follow-up have allowed a more detailed analysis of the cumulated mortality and are presented in this report.

The major part of the studies referred to in this volume has been carried out at the Department of Environmental Hygiene of the Karolinska Institute and of the National Swedish Environment Protection Board (up to 1972 the last mentioned Department was a division of the Swedish National Institute of Public Health). The clinical studies as well as other studies have been carried out in collaboration with the Department of Internal Medicine of the Karolinska Institute at the Serafiner Hospital. The studies on

the US twin registry of the US National Research Council were carried out in 1967 when two of the authors (NC and LF) worked as visiting scientists at the Kettering Laboratory of the University of Cincinnati

The present compilation has been funded as a Special Project of the Council for Tobacco Research USA, Inc. report of which was submitted in February 1977. With some revisions it is now published with support from the Swedish Medical Research Council. The research work has during the years been carried out with grants from different organizations. The formation of the first Swedish twin registry was supported by grants from the Swedish Medical Research Council, the Folksam Insurance Company and the Swedish Tobacco Company upon the recommendation of its medical advisory board. The clinical studies to a great extent have been supported by the National Swedish Association against Heart and Chest Diseases. Since 1969 the major funds for the continuous follow-up of the old Swedish twin registry have come from the American Medical Association Education and Research Foundation. This foundation together with the National Center for Air Pollution Control of the US Public Health Service also funded the studies on the US twin registry in 1967. The formation of the new Swedish twin registry 1971-1975 has been supported entirely with funds from the Research Committee of the National Swedish Environment Protection Board. As of 1975 the Council for Tobacco Research USA is funding the Swedish studies into tobacco and health.

For technical assistance in the preparation of this report the authors are indebted to Ingrid Lundberg BA, Ulla Lörich BA and Margit Dahlquist. Pamela Boston BA has revised the English translation.

2 1 Theoretical Concepts and Models

2 1 1 The phenomenon of twinning

One-egg twins are nature's own contribution to experimentation providing two identical individuals i.e. two human beings of the same genotype. Further, these two individuals have experienced very similar environment before birth as they will during many years in their childhood. The phenomenon of identical twinning is explained by an accidental and immediate partitioning of the fertilized egg cell. This is not too uncommon occurrence but its exact cause is unknown as spontaneous and easily abortions frequently ensue. The birth-rate of one-egg (also called identical monozygotic) twin pairs is fairly constant and does not differ considerably between different population groups. Among Caucasians the rate per thousand full term pregnancies has been estimated at 3.7 (Morton Chung and Mi 1967). There is no conclusive evidence that the phenomenon of monozygotic twinning is related to hereditary factors or other special characteristics of the parents (Bulmer 1960).

Another kind of twin pair develops from two concurrently fertilized egg cells; they are called two-egg twins fraternal or dizygotic. From genetic point of view the partners of dizygotic set are no more similar than ordinary siblings but they do experience much more similar environment both before and after birth than do ordinary siblings. The statistical expectation as to the number of genes they have in common is 0.5 the same as ordinary siblings have.

Dizygotic twins may be of the same sex or of opposite sex. Based on random occurrence the expected number of same-sexed pairs is the same as the number of opposite-sexed pairs. Among the same-sexed pairs the fraction of males corresponds to the sex-ratio which in Sweden is roughly 1.04.

The rates of dizygotic twinning differ considerably between different populations. While the rate of dizygotic twinning in the Caucasian race is on an average 6.6 per thousand pregnancies it is much higher (for example in Nigeria is with rate of about 40 per thousand while it is only about 2) in Japan (Morton Chung and Mi 1967). The explanation is not only racial. It is known that the chance for dizygotic twinning increases with the age of the mother and independent of age also with the number of her pregnancies. Factors that are associated with the social and ethnic environment in observations from England and Wales the total twinning rate ranged from 8-15 per thousand. The lower number representing first pregnancies and the higher representing women who already had given birth to 5 or more children. The chance of having twins as a result of the first pregnancy is little more than 50 percent higher for women over 35 than below that age (Deane and Keane 1972). The rates refer to total twinning but the differences depend on increases of dizygotic pairs.

Multiple births are not confined to twins. The occurrence of triplets is very rare however having rate of about the square of the twinning

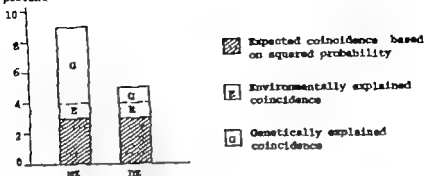
Coincidence Rate
percent

Figure 2.1 Model for Interpretation of the Coincidence Rate
(explanation see text)

The total height of the bars represent the observed coincidence rate and the shaded parts the expected coincidence rate. It appears that the observed coincidence is about twice as high in the monozygotic group compared to the dizygotic. Even for the dizygotic group however the observed coincidence rate is higher than expected. Conventionally the difference $(R+G)$ between observed and expected coincidence measures in both zygosity groups the influence of environmental and genetic factors shared by the two partners. Assuming that the strength of the environmental influence is the same in the monozygotic and dizygotic pairs the difference in G between the total heights of the two bars is due to the genotypic identity of the monozygotes. It has repeatedly been pointed out however that this assumption may not be completely valid. There are indications showing that the members in monozygotic pairs tend to share a more similar environment than do the members in dizygotic pairs (Östlyngen 1949; Smith 1963; Cavalli Sforza and Bodmer 1971; Vandenberg 1976). It can be shown for instance that habitual factors like smoking and drinking are more similar within the monozygotic pairs than within the dizygotic ones (Friberg et al 1959; Kaij 1960; Shiida 1962; Partanen Bruun and Markkanen 1966; Jonsson and Nilsson 1968). This may in itself be an indication of genetic inheritance in regard to these traits but it may just as well solely indicate an environmental effect. An environmental pressure toward conformity whatever the cause this higher degree of similarity has a biasing effect when the hereditary influence on certain disease end-point is evaluated provided the disease is etiologically or genetically related to the habitual factor in question.

In regard to quantitative traits, traits that can be assessed by rank order interval or ratio scales concordance can be assessed in terms of intrapair variances or correlations. Here it is assumed that the variance within monozygotic pairs apart from error variance is solely due to environmental influence while the variance within dizygotic pairs is caused by environmental as well as genetic influence. Several different kinds of heritability indices have been developed. Some criticism has been voiced in regard to the validity of these mea-

rate; quadruplets and quintuplets are very seldom observed. The recent use of hormone-based drugs to enhance fertility by an increased ovarian production has given rise to an increase in the rate of multiple births (Cavalli-Sforza and Bodmer 1971). This is not assumed to affect the rate of monozygotic multiple births however.

Starting with Galton in the nineteenth century the phenomenon of twinning has long been utilized in science for various reasons. The so-called classical twin-method aims at distinguishing between the influence of heredity and environment. The co-twin control method takes advantage of the genetic identity in experimental or semi-experimental designs. Both methods will be presented below.

2.1.2 The classical twin-method

The basic assumption for the classical twin-method is that the two members of a monozygotic twin pair share their environment to the same extent as do the two members of a dizygotic twin-pair. If this were the case any greater resemblance between the two monozygotes as compared to the two dizygotes would be due to the genotypic identity. This concept apparently needs to be bolstered by a definition of resemblance.

One measure of resemblance used for qualitative or quantal traits like disease and no disease is the concordance rate. This rate can be measured in different ways of which the so-called proband concordance is the most general and defined as the proportion of affected individuals among co-twins of previously defined index cases (Allen, Barvald and Shields 1967). This definition has been most used in the literature the main reason being that it estimates the concordance rate in the population which at the same time is equal to the conditional probability that one twin is affected given that the co-twin is affected.

In many studies on twin samples obtained from hospitals or outpatient clinics the investigator will encounter positive cases and he may get in touch with the co-twin through the positive partner. This gives him a sample of twin pairs where at least one member in the pair is positive. The likelihood of finding concordant pairs in this procedure is greater than finding discordant pairs. Appropriate methods of coping with this problem usually based on the proband rate have been worked out. Under some conditions a suitable way of expressing resemblance in such cases is the so-called pairwise concordance defined as the proportion of pairs where both are affected in relation to all pairs where at least one is affected. The influence of incomplete ascertainment on concordance rates has been the subject of several publications (Hrubec 1973; Smith 1974; Hrubec and Allen 1975).

In survey studies based on twin registries compiled on a population basis the question of ascertainment is different. Depending on the efficiency of the compilation procedure such registries may only be more or less complete but the completeness may only be of an insignificant degree depending on the traits under study. In such studies the concordance may be measured as a coincidence rate defined as the ratio of all pairs where both members are positive over all pairs including the negative ones. This rate has been named the coincidence rate (WHO meeting of investigators on methodology of twin studies 1966). It is a straight forward procedure to calculate the expected coincidence rate which is the square of the prevalence rate for a certain trait among all individuals in the twin series. The findings may be illustrated as in figure 2.1.

2 2 The Rationale of the Twin Method in Epidemiology

2 2 1 The basic assumption

In general studies of dose and response cannot be truly valid if they are not performed in "before-after" design or in case-control experiments where cases and controls are chosen randomly. Experimentation has been widely used in medicine and a great variety of methods has been developed (see e.g. Cochran and Cox 1960). The view that only experiments are valid although theoretically perfect defendable has to be considerably modified in epidemiology where with few exceptions experimentation is impossible. To a great extent the epidemiologist has to base his results on the basis of ex post facto experiments the rationale of which is to classify randomly selected individuals into groups of unexposed and already exposed individuals (see e.g. McMahon, Fugh and Ipsen 1960). If exposure is a true random event and not correlated to other risk factors the two groups under study are just as comparable as would be two groups selected on the basis of randomisation procedures. In regard to most exposure factors the concept of random dose induction is questionable particularly for self induced exposures like smoking, drinking and drug consumption. It has been well documented that individuals so exposed show characteristics that the nonexposed do not (cf. section 6). Acquisitions of these habits cannot be regarded random events. It is also often true that the habits go together and are thus not mutually independent. If the influence of such correlated exposures cannot be controlled it has to be nullified by "restriction" which means that all individuals in both groups must be selected as e.g. non-drinkers or nonusers of drugs. Such restriction seems to be easy enough to perform in regard to a few well defined variables but becomes extremely complex if the dose factor is also associated with certain occupations, urban-rural residence differences, physical inactivity and other life-style variables and possibly also psycho-social factors.

In principle multivariate design could be a solution to the problem. In practice however several questionmarks will remain. There may exist correlations to the exposure factor which are not known and thus cannot be controlled. Some influencing factors may be known or assumed but cannot be measured which is the case with most genetic factors.

The importance of controlling for genetic factors depends on how likely it is that such factors play a part in the development of the disease under study and if the same time are associated with the exposure factor and its correlates. In regard to smoking for example the habit can be assumed to be part of a complex constitutionally induced life-style that expresses itself not only in smoking but also in drinking, drug consumption, nightlife, physical inactivity and other habits that can be regarded potentially unhealthy and disease-predisposing. It can further be assumed that this constitutional type in itself and independently of the exposure factor has a genetic predisposition for developing certain diseases. This last assumption has been named the constitutional hypothesis opposed to the causal hypothesis which implies that the exposure factor itself is causally related to the disease.

measurements mainly with a view to the questionable similarity in environmental concordance between monozygotic and dizygotic pairs. A thorough discussion of quantitative concordance measures is given by Cavalli Sforza and Bodmer 1971 and Smith 1974.

2.1.3 The co-twin control method

The co-twin control method is essentially experimental, i.e. focusing on the testing of hypotheses about cause and effect under matching conditions. The validity of any true experiment is due to the fact that the two groups of individuals to be compared, the probands and the controls, if randomly selected, have the same expected mean for any variable to be studied. This makes the two groups ideally comparable but does not decrease the variance within each group. A way to separate out this interindividual variation is to constitute pairs within which as many variables as possible have the same value or are controlled. In all matching the degree of control is dependent on the magnitude of the intrapair correlation, i.e. to what extent the matching variables really have the same values within the pairs. In monozygotic pairs this correlation is unity in regard to genetic factors but may also be assumed to be high in regard to many environmental factors.

The co-twin control method has been widely used in the fields of psychology and education, for example in regard to the learning potential of different teaching methods (see e.g. Gesell 1942; Busén 1953). It has also been used in testing drugs or different treatments in cases where the effect is assumed to be related to individual characteristics with high interindividual variance (Glass 1954; Cerasi and Luft 1967; Alexanderson, Evans and Sjöqvist 1969). The method in its true experimental form has found but little use in epidemiology. A semi-experimental modification of certain potential in epidemiology is to compare differences in response between ex post facto exposed and unexposed partners in series of monozygotic and dizygotic twins. This is the rationale of the twin method in epidemiology and it will be described in detail in the following sections.

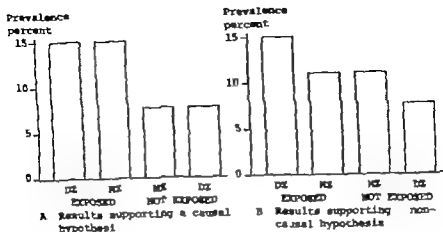


Figure 2.2 The B-series model (explanation see text)

If the trait had been a disease entity figure A would have supported causal and figure B noncausal hypothesis. It should be pointed out that the likelihood of finding complete verification of either hypothesis 1 is small for several reasons. For many exposure factors it may be reasonable to assume that both constitution and the factor each per se or in interaction may have true effect on the disease or mortality entity. The problem is quantitative rather than a qualitative one.

If the trait under study had been competing exposure factor say for example alcohol drinking in smoking discordant pairs an outcome according to A would have demonstrated no control of drinking whatsoever in the comparison within the monozygotes while the outcome according to B would have demonstrated complete control.

The degree of control is of fundamental importance in connection with verification of the causal hypothesis in regard to the exposure factor under study but is of much less importance in connection with possible verification of the constitutional hypothesis. If neither alcohol drinking nor drug consumption is controlled to any extent the discriminating model has lost an important part of its power. It may still be possible to combine the model with restriction or with multivariate analysis. Even if the analysis shows reasonable control of all measured variables there is still possibility that the apparent verification of the causal hypothesis is false because of an unknown and unevaluated competing environmental risk factor correlated to the exposure under study.

The noncausal outcome may also be biased. This would not be due to incomplete control. The major criticism concerns whether the degree of discordance in the exposure factor between the exposed and nonexposed twin partner in the monozygote pair is the same as between the exposed and nonexposed partners in the dizygote pair (cf section 4.6.2).

An outcome in accordance with figure 2.2 B would show that non smokers in smoking discordant monozygote pairs have higher prevalence of the

As genetic factors are completely controlled in monozygotic twin pairs a twin approach may help in discriminating between these two opposing hypotheses provided that enough numbers of pairs discordant for the exposure can be found. Genetic control is not sufficient however if it does not at the same time afford a control of environmental etiologic factors such as the above mentioned habits. Such control cannot be expected to be complete but may be considerable in view of the importance of early childhood for the development of physical and social traits.

2 2 2 The discriminating model

The general approaches to the analysis of empirical twin data are manifold. The following paragraphs will describe and explain the analyses employed in the present report.

First the series of twins is regarded as a number of unrelated individuals distributed in regard to the exposure variable into several categories which if possible are quantitatively assessed. This analysis in the model is named A-series analysis. The statistical evaluation has taken into consideration possible age and sex differences but only to a very limited extent. Other measurable exposure correlates. The A analysis is performed as a confirmation that generally known statistical associations between the exposure factors and disease also exist in the sample studied. The outcome can also be taken as a validation of the measurement methods for both exposure and response variables. As a negative finding could be due to insensitive methods. If such insensitivity can be regarded as unlikely on the basis of other evidence further analysis could still be expected to yield meaningful information if the expected relation between dose and response is hidden or confounded by dose-factors other than the one under study.

The second approach to the analysis is based on monozygotic and dizygotic twin pairs discordant in regard to the exposure variable. This analysis in the model is named B-series analysis. The rationale of the method is to compare the outcome between exposed and nonexposed twins in both the monozygotic and the dizygotic series in regard to the effect variable assuming that the competing influence of confounding variables is decreased especially in the monozygotic series. The decrease of the influence of such confounding variables say drinking behavior is named control and implies an enhanced group comparability. The control may be due to the influence of genetic or environmental factors or both. The results can be displayed as in figures 2 2 A and B.

In each chart (A and B) the exposed individuals from the pairs in the B series analysis are on the left side of the graph and the nonexposed on the right side. The results from the monozygotic twins are displayed in the two middle bars and those from the dizygotic twins in the two outside bars. Figures A and B illustrate two possible extreme outcomes of the analysis. In A there is a notable difference between the exposed and the nonexposed twins in both zygosity series. Whatever genetic or environmental control has been achieved in using monozygotic twins the relationship between the trait under study and the exposure factor is unchanged. Figure B displays the same differences as in figure A between exposed and nonexposed dizygotes. There is however no difference between the exposed and nonexposed monozygotic twins. Whatever genetic or environmental control has occurred there is no statistical association between the exposure factor and the trait under study in the monozygotic series.

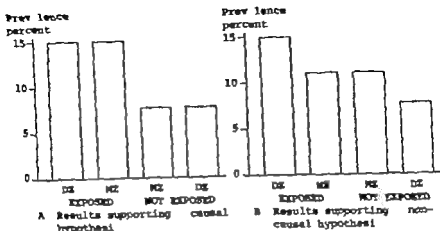


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An outcome in accordance with Figure 2.2 would show that nonsmokers in smoking discordant monozygotic pair have higher prevalence of the

trait in question than do nonsmokers in dizygotic pairs. This is reasonable if one assumes that constitutional factors are of importance for the development of smoking as well as for the trait under study and it would be true even if some difference were found between the smokers and nonsmokers in the smoking discordant monozygotic pairs. One can then also postulate that nonsmokers in general - in the twin series consisting for the most part of concordant nonsmokers - would have still lower prevalence rates as those with a possible smoking constitution would be rare and would influence the prevalence rate to a much lesser extent. A model has been developed in order to elucidate empirically whether or not such prevalence rate differences exist between different groups of nonsmokers.

Within each zygosity group all nonsmoking individuals can be regarded as a finite population of nonsmokers. As any parameter e.g. the prevalence rate of excessive alcohol drinking is known within sex and age groups in this finite population it is possible to calculate an expected mean and variance for any randomly chosen sample of a certain size and with a given sex and age distribution. If a sample is not randomly chosen but selected in regard to a certain single criterion that is assumed to be unrelated to the item under study the sample mean under this assumption will be an unbiased estimate of the population parameter and subject to random variation only. Consequently a significant difference between the sample mean and the sex and age standardized expected value would require the rejection of the hypothesis that the selection criterion and the trait under study are unrelated.

This reasoning leads to the following mode of analysis. Monozygotic and dizygotic twins were treated separately throughout. All nonsmoking twins in each zygosity group were assumed to constitute a nonsmoking population. Prevalences for each variable were calculated within sex and five-year age groups. Among these nonsmokers several samples were drawn on the basis of the smoking status of the co-twins. In this way one sample was formed by the nonsmoking partners of present cigarette smokers, another sample by the partners of all present smokers, and a third group was constituted by the partners of former smoker. A fourth group was formed by the nonsmokers who had a nonsmoking partner, i.e. one twin was randomly selected from all nonsmoking concordant pairs. On the basis of the sex and age structure within these samples and the prevalence rates in the population expected values were calculated. The differences between the observed and the expected values were then statistically evaluated. The analysis has been named NET-analysis in the following as it is based on Non Exposed Twins.

3 TWIN REGISTRIES AND TWIN STUDIES

3.1 Twin Registries in Epidemiology

3.1.1 Availability of twins

As stated above in most western countries about two percent of all individuals are born members of twin pair. If survival time were the same for twins as for singletons - which is not altogether true - the Swedish population should comprise 160 000 twins and the US population more than 4 million. The twin approach in epidemiology requires in most cases however that the twin pairs should be unbroken by death at the start of the investigation. This decreases substantially the target population and when other selection criteria have to be met like those of including only same-sexed twins or twins with discordant environmental exposure the target groups become quite small.

Most western countries make separate tabulations for twins in their vital statistics on births. To our knowledge however the fact of being born a twin is seldom carried further in population registries. Special methods have to be developed for compiling twin registries.

Many clinical and genetical studies on twins have been performed on samples the selection of which is closely related to the trait under study. For example in studies of the inheritance of diabetes mellitus each person admitted to hospitals or outpatient clinics operating within certain research areas is questioned as to whether or not he belongs to a twin pair. If he does the twin partner is located through the disease-bearing twin. This compilation procedure is not valid in connection with such epidemiological research where the main design has to be of the prospective type. It is therefore necessary to define the target stratum within the population and to arrive at complete ascertainment of all twin individuals belonging to that stratum. Three procedures may be considered.

The most direct procedure is to ask each individual within the stratum whether he is a twin or not. Assuming total and true responses to this question the sample has to be regarded as completely ascertained. Such screening could take place in connection with population census based on individual reporting. It is obvious however that the procedure will involve questioning 50 times as many individuals as will be utilized for the study. Another method is to use individual birth records as starting point to identify the twin births by name or number and to try to locate them in the general population. This seems to be an ideal procedure provided that each individual were given a unique number at birth which he kept throughout his life and further that the registry of the current population contained this number and that the investigator had access to it. This is presently the case in Sweden but the procedure has been operating for only little more than 10 years and thus it is not helpful in locating twins born before that date.

Neither of these twin procedures gives any information on what has happened to the twins that were not found. The third procedure which is rather complicated and can be used in very few countries is to follow up the individual from the parish or registration area in which he was born by tracing his movements from place to place until he finally is found either deceased or living at a certain address. This process will also furnish data about the migration pattern - even emigration to a certain point - and gives statistics in regard to mortality. These two last mentioned procedures were combined in the compilation of the Swedish twin registries.

Other procedures of unknown merit - but probably relatively unreliable in regard to completeness - are to advertize for twin pairs in news papers, magazines, television etc.

3.1.2 The WHO survey

As has appeared from the above section the availability of twins is quite restricted in small countries. Because of the numerous variables that have to be used as group defining criteria epidemiological analysis requires a large number of individuals. One way to enhance the possibilities of a meaningful analysis would be to seek cooperation with other countries where twin registries are under operation. To this end the World Health Organization in 1965 (WHO meeting of investigator 1966) made a survey by sending a mailed questionnaire to researchers who were known to the Organization to be involved in twin studies. The result of the survey has been summarized as follows:

Out of 39 reported twin series covering in all about 60 different ongoing or planned research projects about two thirds are of the selected type i.e. obtained from hospitals, clinics, school etc. The remaining registries cover whole populations or well defined parts of a population; about one third of these however contain data obtained from birth records only. The twin series vary in size from 40 to 50 pairs to more than 20 000; the smaller series mostly consist of case studies and the larger complete populations. Twin series of the non selected type and large enough for use in epidemiological investigations are available mainly in the USA and in the Scandinavian countries. Some of these include current addresses of twins others do not at present but comprise a large number of twins and must be looked upon as very important sources for epidemiological work.

This was in 1965. Large scale epidemiological studies on twins outside Scandinavia and the USA have not hitherto been reported but some are under way in Finland and others might be expected from other countries during coming decades.

3.1.3 Operating registries

The first fairly large scale epidemiological investigation of twins concerning genetics and the epidemiology of chronic diseases is related to smoking were published by Danish researchers (Haug et al. 1968; Harvald and Haug 1970; Haug, Harvald and Reid 1970). Data are based on a registry that was set up in 1954 comprising about 7 000 sets of twins of which 2 500 were of different sex.

The Swedish twin registry began its operation in 1959 and the first reports began to appear in the early 60. The registry covers about 11 000 same-sexed twin pairs in the cohorts 1886-1925. It covers both males and female and has mainly been used for studies concerning smoking morbidity and mortality. Some clinical studies on subsamples have specifically focused on respiratory and coronary disease.

The Swedish twin registry was extended in 1971 to cover another 15 000 same-sexed twin pairs in the cohorts 1926-1958. The studies on this extension of the registry in addition to the above mentioned smoking related studies will be used for investigating the impact of the environment in general.

In the US twin registry was set up from birth certificates and records of the Veterans Administration in the beginning of the 60 and has since then been maintained by the National Academy of Sciences (Jablott et al 1967). An epidemiological study using mailed questionnaire was carried out in 1967 covering about 4 400 male twin pairs. This study in collaboration with the Department of Environmental Hygiene also focused on medical problems connected with tobacco smoking. The registry has since been used for other studies.

The Swedish and US twin studies will be described in detail in the following sections.

3.1.4 Aspects for future registries

As mentioned above international cooperation seems to be important and necessary to furnish enough material for studying the effect of environmental exposures on twin pairs discordant for such exposures. In order to find support for these endeavors and to discuss problems of coordination and standardization international meetings have been convened upon three occasions.

The first meeting organized by the World Health Organization and mentioned above (WHO meeting of investigators 1966) considered methodological problems of twin studies in regard to determination of zygosity, sampling techniques, organization and maintenance of twin registries and analysis and presentation of results. The meeting agreed that cooperation efforts should be made in order to assist comparison between existing and future twin studies. The group stressed the need for samples of considerable size in order to obtain discordant twins for comparative analyses of environmental effects in chronic conditions.

The second meeting was set up under the sponsorship of the Department of Environmental Hygiene of the Karolinska Institute and was held in Porto Rico in December 1969 (Twin Registries in the Study of Chronic Disease 1971). Apart from thorough evaluation of results hitherto obtained from twin studies on smoking and health the main goal of the meeting was to establish standardized procedures for survey investigations as well as standardized measuring techniques possible to apply in epidemiological fields. It was the consensus of the convened group that twin studies should be conducted in variety of populations in different geographical and ethnic settings. It was further stressed that to interpret the effect of smoking and disease it will be essential to collect data on other living patterns and environmental factors as well as personality type, behavior and the more commonly recognized risk factors.

After the meeting in Puerto Rico and with financial support from the Council for Tobacco Research site visits were made at various research centers in order to stimulate the interest in twin research and to seek cooperation for performing extensive studies on environment and health using twin samples. As a result of these efforts a number of researchers convened at a meeting at Miami Beach in 1972. The meeting strongly emphasized the need for standardized methods and that a common questionnaire should be worked out. The interest in cooperation was great but the possibilities of effectively establishing registries in several places were not good enough to recommend an undertaking at that time. However the Institute of Hygiene in Helsinki, Finland, proved to have excellent possibilities of compiling a Finnish twin registry to cover 10 000-15 000 pairs. The registry is now on its way and it may be followed by several others. A twin research program has also been initiated at the Kaiser Foundation, the Permanente Medical Group, California, USA, which will cover close to 8 000 pairs.

3 2 The Swedish and US Twin Studies

3 2 1 Target populations

The Swedish twin studies have been performed on two target populations differing only by year of birth and year of compilation. The first target population was defined as male and female same-sexed twin pairs born 1886-1925 and still living within the country as unbroken pairs in 1961. The year of compilation. The second target population was defined in the same way but comprised pairs born in 1926-1967 and unbroken at the time of compilation in 1973. Emigrants from or immigrants to Sweden are not covered by the definition.

The target population in the US study was defined as male twin pairs born in 1917-1927 where both members had served in the US Armed Forces as ascertained through the Veterans Administration. All except 9 states in the continental US were covered.

The target populations in the Swedish and the US studies differ considerably. While the Swedish target population in comparison with the total Swedish population is biased only in regard to the requirement of concordant survival up to the day of compilation, the US study is restricted to a special population group. The requirements for registration with Veterans Administration imply that both members of the pair were physically fit at the time of the induction into the Army.

3 2 2 Compilation procedures

The first step in the compilation of the older part of the Swedish registry was scanning of all birth registries to find birth records giving date and place of birth, family name and the first names of the twins' parents. This procedure involved scanning of about 5 million entries and yielded a total number of same-sexed pairs amounting to about 41 000 liveborn pairs, a number that deviated less than 0.1 per cent from the official statistics on twin births during the period.

The second step in the procedure was to arrange the initial file by county and birth date. Mail lists to each of the 29 counties in Sweden and to ask the registries office to give information about twins born in the county. In this way, all twins still living in their county of birth were located. At this stage the current addresses of one or both partners of about 16 000 pairs were obtained. A twin pair was considered located as soon as the address of one partner was obtained. An expected large number of pairs subsequently had to be discarded because the non-located partner turned out to be dead.

Following this second step in the compilation procedure, about 60 percent of the original number of twin pairs remained to be traced. Of this remainder, it could be expected that many of the individuals had died in their birth parish. Thus the remainder of the file was arranged by birth parish and lists were distributed to all 2 000 or so parishes asking the parish office to return information on the twin pairs on the list stating whether they had died in the parish or had moved to another parish. Again, in cases of death the pair was discarded from the file. In cases of migration to another parish, the new parish was asked for information about death or migration and so on. In this way the remainder

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3 2 2 Compilation procedures

The first step in the compilation of the older part of the Swedish registry was scanning of all birth registries to find birth records giving date and place of birth, family name and the first names of the twins' parents. This procedure involved scanning of about 5 million entries and yielded a total number of same-sexed pairs amounting to about 41 000 liveborn pairs, a number that deviated less than 1 per cent from the official statistics on twin births during the period.

The second step in the procedure was to arrange the initial file by county and birth, to mail lists to each of the 25 counties in Sweden and to ask the registrar's office to give information about twins born in the county. In this way, 11 twins still living in their county of birth were located. In this stage, the current addresses of one or both partners in about 16 000 pairs were obtained. A twin pair was considered located as soon as the address of one partner was obtained. As expected, a large number of pairs subsequently had to be discarded because the non-located partner turned out to be dead.

Following this second step in the compilation procedure, about 60 percent of the original number of twin pairs remained to be traced. Of this remainder, it could be expected that many of the individuals had died in their birth parish. Thus, the remainder of the file was arranged by birth parish and lists were distributed to 11 200 or so parishes asking the parish officers to return information on the twin pairs on the list, stating whether they had died in the parish or had moved to another parish. Again, in cases where the pair was discarded from the file, in cases of migration to another parish, the new parish was asked for information about death or migration and so on. In this way, the remainder

of untraced twins gradually became smaller. At a stage when less than 15 percent remained untraced, it was decided to stop these procedures. New lists covering the total remainder were then sent to all 25 counties. After this step in the procedure there was still a remainder, numbering slightly under 2 000 pairs. Once again the parish officers had to be contacted. Finally, about 1 000 of the original 41 000 pairs had to be discarded as untraceable.

The compilation was not yet complete. The located twin partners in all pairs from which only one address had been obtained had to be contacted. The number of such cases was around 14 000. About 8 000 of these twins reported that their co-twin was dead, about 5 000 reported the address of the co-twin and about 1 000 failed to return the questionnaire. Finally a total number of 12 889 pairs had been located with known addresses of both partners. It could be calculated that this number of pairs covered at least 91 percent and probably more than 95 percent of the actual target population defined above.

It should be mentioned that this compilation procedure was complicated, expensive and time-consuming. It was performed during a time when the Swedish population registry was not yet computerized and only few of the individuals had a unique birth number. It is not possible to tell how significantly the twin series obtained deviates from the actual target population. Most probably a large number of the missing individuals were dead or had emigrated. In many cases, however, they may have been institutionalized or may belong to the category of "missing persons" who for unknown but probably social reasons escaped the registration by the authorities.

The second part of the Swedish twin registry set up in the beginning of the 70's was compiled at a time when the Swedish population registry was fully computerized. The first step of the compilation procedure, namely the screening of the birth records, had to be the same, however, since the data record on tape did not contain information about twins. Opposite-sexed pairs were also included in the compilation base of the study. According to official statistics, 44 636 pairs were recorded as liveborn in complete pairs. This initial file could then be computer matched to the registry of the total living population. The procedure was not quite straightforward, though, as a large number of the twins born before 1950 did not have a unique birth number. The difficulties were overcome by using other characteristics like birth county, initial of family and given names etc. The resulting final number of twin pairs available for study amounted to 40 991 pairs.

The efficiency of the compilation procedure used for the new twin registry was better than that for the old one in regard to cost and time elements. It seems also to have been efficient in regard to yield. Among those not located according to the procedure described, 50 individuals were selected and processed according to the same procedure as had been followed for the old registry. 44 were found to be dead, 3 had emigrated and 3 were found to be living within the county (Medlund et al. 1977). Although this sample is small, the yield agrees fairly well with the yield of 90-95 percent of the pairs estimated as located by the compilation procedure for the old registry.

The US registry was also established from birth certificates. The target population was all white male multiple births in the years 1917-1927, with parent's states of the continental US (Jablon et al. 1967). Of

Table 3.1 Number of Conjoint Pairs in the 1961 Questionnaire Study on the Older Cohorts of Swedish Twins by Sex, Age and Zygosity

Year of Birth	DIZYGOTES		MONOZYGOTES	
	Males	Females	Males	Females
1886-1895	218	368	190	212
1896-1905	576	810	330	405
1906-1915	1009	1228	526	646
1916-1925	1181	1442	603	744
Total	2984	3858	1649	2007

The approximately 54 000 pairs identified in this way about 16 000 pairs were found in the Master Index file of the Veterans Administration indicating that both members of these pairs had undergone military service. Service personnel and medical records were abstracted for the latter group to determine medical histories during the service period and get information on physical characteristics that are helpful in classifying zygosity. Survival of members of these 16 000 pairs was monitored through record resources of the Veterans Administration. Pilot evaluation indicated that mortality ascertainment is about 97 percent complete. Medical histories have been updated through questionnaires and from records of the Veterans Administration for those obtaining medical care through that organization.

3.2.3 The questionnaire surveys

The first Swedish questionnaire was mailed 1961 and was to some extent part of the compilation procedure. It was also used to obtain the address of the co-twin in pairs where only one twin had been located. Further, the questionnaire asked for information about smoking habits, residential history in very broad terms, some sociological background variables such as education and marital status and series of questions pertaining to the diagnosis of zygosity. Detail on the diagnosis will be given in section 3.3. Out of the total number of 12 889 pairs available for study 23 429 individuals returned their questionnaires corresponding to a response rate of 81 percent.

Of primary interest however is the number of pairs where both partners have returned their questionnaires. These figures distributed by sex, age and zygosity are given in table 3.1 above.

The nonresponse rate counted in pairs amounted on an average to 15.1 percent and was by and large the same for all sex and age groups with a possible tendency to higher nonresponse in the younger groups. It appeared that zygosity could not be determined in 439 pairs or 4.0 percent.

A second questionnaire was mailed about a year after the first survey had been completed. This questionnaire contained medical as well as sociological information. Apart from some questions pertaining to the past history of disease experience and hospitalization, the questionnaire focused on present symptoms in regard to respiratory and cardio-

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Tabl 3 2 Number of Conpl te Pairs in the 1973 Questionnaire Study on the Younger Cohorte of Swedish Twins by Sex Age and Zygosity

Year of Birth	DIZYGOTES		MONOZYGOTES	
	Males	Females	Males	Females
1926-1935	823	1063	516	611
1936-1945	1116	1304	718	880
1946-1954	1764	1803	1058	1242
Total	3703	4170	2292	2733

twins in the same fashion as in the Swedish studies Out of mailing to about 21 000 individuals replies were obtained from both partners in 7 372 pairs This group was defined as target group for second questionnaire mailing

The second questionnaire contained about the same information as the Swedish 1967/70 questionnaire Most questions were translations of those used i Sweden The intention was to duplicate and to supplement the Swedish study Complete replies from both members f the pairs were obtained for 4 379 twin pairs f which 1 876 were monozygoti

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3 2 4 The clinical examinations

During the course of the twin research program five special studies have been pe f rmed among which four have utilized subsamples from the twin registry f clinical examinations The fifth special study focused on psycho-social problems in connection with coronary heart disease and was based partly on the questionnaire surveys and partly on clinical investi g tions on samples from the general population

Results from these five special investigations will appear throughout the present report This section will present an overview of the aim of the different investigations and detailed account of the construction f the subsamples from the twin registry or other samples obtained from the gener l population The special investigations will here be presented in chronological rde

The primary object f the clinical investigation by Lundman (1966) was t examine the association between smoking and coronary heart disease Lung function was iso examined mainly with view to locating whether the association between smoking and lung function would be detected when the constitutional factor were controlled Further Lundman wanted to evaluate whethe any association between smoking and coronary heart disease could be diagnosed on the basis f case history and lactrocar diographi examinations; whether the acute lev tion in blood pressure used by nicotine would result in changes in blood pressure over long period; and lastly whethe genetl factor influence the development f coronary he rt disease

vascular disease. The sociological variables aimed at comparing different survey groups in regard mainly to socioeconomic factors. The twins were asked about marital status, number of siblings and children, employment status and education.

This second questionnaire was mailed to all twin pairs in which both partners had returned the first questionnaire, i.e. 10 947 pairs or 21 894 individuals. Returns were obtained from 20 083 individuals or 92 percent. The series of complete pairs to be used in the epidemiological study amounted totally to 9 319 pairs or 85 percent.

It shall be noted that the nonresponse occurring between the first and second questionnaire to some degree was due to death occurring between the two mailings.

A third questionnaire study on the older Swedish twin registry was carried out in 1967. This questionnaire aimed at a closer description of the twin-individuals in terms of habitual factors other than smoking. It contained questions about drinking habits, food habits, physical exercise during work and during leisure time and further questions about working conditions such as change of employment and employer, overtime and extra work etc. Questions about smoking habits as well as symptoms referring to respiratory and cardiovascular disease were repeated with the same phrasing as in the second questionnaire.

The questionnaire was mailed to 10 451 living twin pairs. Returns from both members of the pairs containing complete information on smoking habits were obtained from only 6 130 pairs, constituting 58.7 percent of the total number of pairs eligible for the questionnaire. A follow-up on pairs who had not answered the questionnaire in 1967 was performed in 1970 and embraced pairs who were still unbroken at that time. The nonresponse rate was partly due to mortality between the two last mentioned points of time.

The second or "new" part of the twin registry, i.e. pairs born between 1926 and 1967 up to now (1977) has been utilized for one questionnaire study only, covering same-sexed pairs born between 1926 and 1958. The questionnaire was quite comprehensive, in addition to most items of the 3 questionnaires mailed to the first part of the registry, it covered a series of questions pertaining to the individual's adjustment to society in psychological terms and questions about drug consumption in terms of tranquilizers and sleeping pills etc. Data were also compiled in regard to a detailed residential history, type of work and working conditions, attitudes about the general environment in terms of annoyance reactions caused by factors such as odors, dust or noise at the place of work or place of residence.

This questionnaire was mailed to 21 147 complete twin pairs. The number of complete twin pairs responding to the questionnaires is presented by sex, age and zygosity in table 3.2.

The twin pairs in the US National Research Council Registry were approached with two questionnaires. The first was mailed to 22 000 individuals and contained questions about similarities of the twins as children. It also determined the medical history since completion of military service. The replies to this questionnaire were used to determine zygosity of the

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Total	3703	4170	2292	2733

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Lundman utilized a subsample from the twin registry and the criteria for selection were the following: The twin pairs should be concordant in respect to urban or rural environment and discordant with respect to smoking habits. All the monozygotes were selected at random with respect to age and sex whereafter the same number of dizygotes was chosen with corresponding age and sex distribution. All pairs were selected as concordantly living in metropolitan area. Lundman invited 247 twin pairs fulfilling these criteria of selection to participate in the study. Out of these 196 (80 percent) were examined. Of these pairs 92 were monozygotic and 104 dizygotic and they ranged in age from 38 to 77.

The reasons for nonresponse are extensively discussed by the author who concludes that the nonresponse did not affect the end results to any appreciable extent.

In addition to a sociological interview concerning a more detailed description of the subjects' smoking habits and a medical interview focusing on respiratory and cardiovascular symptoms, a series of objective characteristics were measured: physical examination including anthropometric measurements such as skinfold thickness, skeletal length and breadth, muscular power, weight and height and blood pressure; laboratory tests including blood and urine tests, x-ray of heart and lung, lung function tests such as dynamic spirometry and nitrogen wash out delay, electrocardiographic examinations during rest and work and working capacity. Further in cooperation with Blomstrand (Blomstrand and Lundman 1966) laboratory examinations were performed in regard to blood contents of cholesterol, triglycerides and phospholipids.

A study by Liljefera (1970) focused on hereditary and environmental factors in pairs concordant and discordant in regard to coronary heart disease. The purpose of his study was to examine the concordance rate for the various manifestations of CHD in MZ and DZ pairs selected from a population of male twin of working age and to study in discordant pairs intrinsic factors associated with CHD including anthropometric factors, blood pressure, lipids and uric acid in serum, diabetes mellitus and extrinsic factors such as smoking, physical activity and psychosocial variables. Liljefera used a subsample from the first Swedish twin registry and his criteria for selection were the following: The crude selection consisted of twins from the base material who according to the questionnaire in 1967 were considered to have infarction, angina pectoris. Such individuals numbered 189 in 170 pairs. To reduce the risk of false positives a preliminary appraisal of the symptoms of angina pectoris reported in the questionnaire was made. This appraisal was done by telephone but in some cases by personal interview. Out of these 189 persons 70 described symptoms that were not considered indicative of coronary heart disease and another three cases were not accessible for study which left 116 persons for study distributed over 104 pairs. Another 13 pairs had to be discarded as the one twin was not able to be examined.

The Myrhed study was published in 1974 and centered on alcohol consumption in relation to factors associated with coronary heart disease. He emphasized that in most earlier studies great interest has been devoted to the relationship between excessive alcohol consumption and specific somatic response but that he wanted to examine the extent to which moderate long standing alcohol consumption is accompanied by somatic deteriorations and if it gives rise to factors associated with IHD.

The material for the Myrback study was selected on the basis of reports on alcohol consumption in the 1967 questionnaire. Certain discordance criteria in regard to alcohol consumption were also maintained. A total of 92 male pairs aged 45-65 years were invited to participate in the study. 70 complete pairs were examined among whom 14 were monozygotic and 56 dizygotic. No female pairs were included in the study. As in the other medical examinations, a sociological interview focused on alcohol consumption and purported to confirm the data obtained from the questionnaire survey. The medical interview concerned respiratory symptoms such as cough and phlegm, asthma and dyspnea, as well as cardiovascular symptoms according to the full questionnaire of the London School of Hygiene and Tropical Medicine. The diagnosis myocardial infarction was based on the medical interview as well as on electrocardiography and serum enzyme indicators of liver damage were made.

The study by Floderus (1974) focused on psychosocial status in relation to coronary heart disease and associated disorder. The purpose of the study was to investigate whether or not factors associated with the psychosocial status of the individual were related to premature death and specifically to death by coronary heart disease. At the same time the study elucidated the association between these psychosocial factors and behavior characteristics such as smoking and drinking as well as other previously documented risk factors in the development of coronary heart disease. The study employed the 1967 twin questionnaire results as well as results obtained from studies on other samples from the Swedish population. A most specific aim of the study was to develop a psychometric scale indicative of psychosocial disorder in regard to the dimensions of lability-instability and introversion-extroversion.

The special study groups consisted of samples from the populations of Gothenburg and Boden comprising 394 men born 1914-1928. In this study group the variables investigated apart from the psychosocial scale developed consisted of smoking, physical activity, including heart rate, overweight, cholesterol and triglyceride level, blood pressure and diabetes. Coronary heart disease was represented by angina pectoris, severe chest pain for more than 10 minutes, myocardial infarction and family history of coronary heart disease.

Another subject group consisted of a random sample of about 1,200 men and women, 25 to 45 years of age, selected from the Stockholm area and county in the southwest of Sweden. The nonresponse rate in this investigation performed via mailed questionnaire was 19 percent and the group investigated comprised 975 subjects.

In addition to the psychological scale the variables investigated comprised factors of risk for the development of coronary heart disease, namely smoking, alcohol consumption, lack of physical activity and overweight. Information on use of tranquilizers and sleeping pills was also obtained and related to psychosocial instability as a validity test of the instability classification.

The fifth special study was performed by de Faire (1974) and dwelled upon ischemic heart disease as death discordant twins. The specific objectives of the study were to evaluate the genetic influence on coronary heart disease through associations between the occurrence of coronary heart disease in the surviving co-twin and the cause of death of

the partner. He further studied associations between the risk factor profile in the surviving co-twin and the cause of death of the partner and lastly environmental differences in the death discordant pairs as elucidated from earlier questionnaires.

The target population was constituted by pairs from the old twin registry born 1901 and later where one member of the pair died during January 1 1971 through March 15 1973 and the other member at that time was still alive. A total of 262 male and female twin pairs below the age of 60 had become death discordant i.e. one member in a hitherto unbroken pair died during the period. All the co-twins to the deceased twins were invited to take part in a medical examination. Out of the 262 twins invited 205 agreed to be examined giving a response rate of 78.2.

Apart from a sociological questionnaire about smoking and drinking among other things questions were posed in regard to symptoms of coronary heart disease. The physical examination comprised blood pressure measurement and anthropometric measurements such as weight height and skinfold thickness. Electrocardiographic examinations were performed during rest and for nearly all the subjects during and after an ergometer exercise test. Further x-ray examinations were performed as well as laboratory blood investigations in regard to cholesterol triglycerides and uric acid.

In summary these special investigations - apart from the Lundman study that also considered respiratory symptoms - have all focused on risk factors for coronary heart disease. Smoking has been the main item of investigation in one study while the others have focused on several other risk factors of coronary heart disease.

Zygosity Determination

Zygosity can be determined with a high degree of reliability from evaluations of serological characteristics. Anthropometric measurements such as fingerprint ridge counts can also be used. The more measurements carried out on each twin pair, the smaller is the chance that the diagnosis is in error. By considering the prevalence of the specific genetic marker in the population, their exact probabilities can be estimated as to the degree of reliability. These measurements however require a personal contact with the individual to obtain blood samples or fingerprints. To determine zygosity in small samples and for exact assessment of special pairs, such methods may be necessary. However, on large samples such as are needed for epidemiological studies, the exactness of the serological method can not be defended in consideration of the exorbitant costs involved. In epidemiology furthermore it is not absolutely necessary to avoid every error of a somewhat less precise method.

An alternative to the serological or anthropometric method for determining zygosity is an assessment of the physical resemblance of the two twins in a pair. It has been pointed out that this resemblance can be very striking even in dizygotic pairs, since a certain proportion of dizygotic pairs have more than half of their genes in common. This type of error has to be accepted, possible, but nevertheless it has to be regarded as occurring seldom. In large scale study the degree of resemblance between the two twins can be assumed to be reliable enough for zygosity classification.

The resemblance between the two partners of a pair can be judged by inspecting photographs taken in their childhood or at a later point in time. It was deemed sufficient however to ask both partners in a twin pair whether they regarded themselves to have been very alike as children. This has been the approach in both the Swedish and the US twin studies and the equivalent of the expression "as alike as two peas in a pod" was believed to be a significant indication of monozygosity (Cade 1961; Jallón et al 1967).

Two alternative responses to the "peas-in-the-pod" question were offered in the mailed questionnaire. Statements from both members of the same twin pair that "as children they were as alike as two peas in a pod" classified them as monozygotic. If both members checked that they were only of family likeness, the pair was regarded as dizygotic. If the partners in the pair did not agree in their answer, no diagnosis was made.

At an early stage in the Swedish twin program, mailed questionnaires containing the zygosity items were sent to a regional sample of located twins. A random sample of 200 pairs where both members had returned their questionnaire was selected for serological analysis. The selection was independent of replies to specific questions. The serological examinations comprised five independent systems, namely A, A₂, B, Rh₀ (determined by anti-C, anti-D, anti-E, anti-c and anti-e), haptoglobin and for those twins who were concordant in the first four systems, the Gm-serum. Well. All determinations were performed in

accordance with the standard procedure at the laboratories in question¹. On the basis of prevalence of these in the general population the median probability of concordant pairs being monozygotic was estimated as 96 percent.

The requirement of agreement within the pairs in regard to the question of "being as alike as two peas in a pod" or being of family likeness only led to the omission of 14 pairs. According to the questionnaire diagnosis 72 pairs could be regarded as monozygotic and 114 pairs as dizygotic. Among the first mentioned 72 pairs 71 were also monozygotic according to the serological determination and among the last mentioned 114 pairs 104 were dizygotic according to the objective tests. This implies quite a high rate of confirmation which is the most important validity criterion in an epidemiological investigation of the type intended.

On the basis of these results the questionnaire method was found to be reliable enough for an epidemiological investigation. A similar procedure was followed in the US investigation. The results showed by and large the same degree of reliability.

In the Swedish special investigation mentioned above the results of the questionnaire diagnoses were further validated. In the Lundman study zygosity checks were made on the selected pairs using the A₂A₂B₀ and haptoglobin systems for all pairs diagnosed in the registry. A more thorough serological examination in which the MN and Rh-systems were also included was made on the pairs who were not diagnosed in the twin registry. Further color photographs were taken of most of the subjects included in the study. All but two out of 93 pairs diagnosed as monozygotic in the twin registry were so classed by these methods. All 98 classed as dizygotic in the twin registry were so classed on the basis of the above method although some of them displayed blood group and photographic similarity. It again should be mentioned that the blood grouping procedure in this subsample did not include as many genetic markers as were included in the validity examination in the Swedish twin registry.

Myrbed (1974) also made an extensive serological examination on the pairs included in his study. All of the 54 pairs initially diagnosed as dizygotic had the same zygosity according to serological examination and 14 of the 16 pairs initially diagnosed as monozygotic proved to have been correctly diagnosed.

In summary the classification of zygosity employed in these studies can not be considered to be far from the true values. The most likely bias would be that traumatic events before or after birth would tend to make monozygotic pairs different and thus cause them to categorize themselves as dizygotic. The discriminating power of the twin model would of course hereby be decreased. More serious is the possibility of classifying dizygotic pairs as monozygotic. Anyhow this error of classification would also tend to decrease the difference in genetic discrimination between the two zygosity groups and thus unfavorably affect the possibilities of an empirical statistical difference.

1) The blood examinations as to A₂A₂B₀ MN Rhstatus and haptoglobin were performed at the Institute for Medical Genetics, University of Uppsala under the supervision of L. Beckman PhD. The Gm-Determination was performed by R. Grann MD. The Department of Bacteriology Univer-

CONCEPTUAL CONSIDERATIONS AND CLASSIFICATION OF VARIABLES

4.1 The Concept of Dose and Response

Epidemiology has adopted its own vocabulary and especially in discussions of dose and response expressions are used among which risk factors precipitating factors and intervening factor are common but not always clearly defined.

Presented with a disease of unknown etiology the researcher's first step is almost always retrospective. This implies comparing groups composed of people with and without the disease. The whole panorama of possible causes is studied in the hope of finding some factors that are more prevalent among the diseased people as compared to the healthy. Although invaluable in epidemiology the retrospective design does not have the power of its own to point out smoking and drinking as causative agents nor even as risk factors. Other factors not known, not measurable, may theoretically be the real cause and an apparent association between smoking, drinking and disease may be spurious or caused by high correlation between the real factor and the apparent one.

Prospective research gives rise to another situation. A population of healthy individuals may be subdivided in regard to presumed causative agent. If subsequently occurring specific morbidity or mortality turns out to be higher in the group where the presumed causative agent is present than in the other group, this agent is defined as a "risk factor". The prospective approach makes it possible to test hypotheses regarding the relationship between risk factor and disease but based only on such data and without supporting evidence it is still not theoretically possible to prove causality for the same reasons as have been mentioned above in regard to the retrospective design.

Risk factors can be external or internal. Typical examples of external risk factors are habit like smoking and drinking. Such factors can be named exposure factors or dose. Other external risk factors are occupational exposures to gases and dusts, general air pollution, drug consumption, inadequate nutrition, physical inactivity, heavy work load, etc. Among internal risk factors for coronary heart diseases are obesity, high blood pressure, increased serum content of lipids such as cholesterol (Mörck 1975; Keys 1975; Werko 1976). The internal risk factors cannot be regarded as exposure or dose. They are all biological conditions that either may have genetic origin or may in their turn have been caused by external exposure factors. A good example is increased serum cholesterol which is influenced by genetic factors but also depends to a large extent on food habits (Osborn et al. 1959; Meyer 1962). In this sense increased cholesterol is partly an effect factor.

The concept of intervening or intermediary factors is not easily defined. It can be hypothesized that "personality" is an internal risk factor for coronary heart disease. Type A and type B behavior are well documented psychological correlates to smoking as well as to coronary heart disease (Friedman and Roseman 1959). Personality is of course

a very wide concept. It has been mentioned in earlier sections for instance that smokers and nonsmokers may represent different personality types. The smoker's personality is characterized not only by smoking but by a series of other habits like drinking, drug and physical inactivity (cf. section 6.2). If this personality independent of the risk factors it involves is causally linked to coronary heart disease, the risk factors may play no other part in the etiology complex than simply being signs or symptoms of this personality. They may however just as well play an important role of their own in this psychologically determined environment and may then be regarded as precipitating factors.

A few words will also be said about feedback mechanisms. A risk factor like smoking may cause a certain disease. The awareness of the disease may give rise to maladjustment, sleeplessness and other psychological conditions which then in the analysis will show up as correlated with smoking. The disease itself is in this way intervening in the relationship and there may be no direct causal link between the mentioned psychological conditions and smoking. The interpretation of relations of this kind is even more difficult to make in cases where the disease condition is subclinical and has not been recorded as a manifest disease. An example is physical inactivity; certainly nobody would question that a patient with anginal troubles would cease in physical activity long before the disease condition is clearly known to himself.

Against the background of these considerations, an attempt to classify all variables in terms of dose and response is rather futile. Arbitrarily the authors of the present report have chosen to name external risk factors of the habitual type as dose variables and symptoms and signs of disease or mortality as medical effect variables. Other variables like socio-economic or psychosocial status have been named covariables as they have been used mainly for control. No consideration has been given to the fact that they sometimes may play the role of an internal dose factor or of an intermediate effect.

The Concept of Discordance

In section 2 1 2 the concept of concordance was discussed as means of evaluating possible genetic influence. It was mentioned that concordance could be measured with regard to both qualitative and quantitative traits. Concordance measurement is an instrument of the classical twin method.

In the co-twin control method as it is modified for epidemiological evaluation discordance is a fundamental concept. In focusing for instance on smoking habits the very target for the twin analysis is pairs the members of which have dissimilar smoking habits. One clearcut grouping is nonsmokers versus smokers and this qualitative classification may be further strengthened by requiring that the smoker should be regular smoker cigarette smoker or smoker of more than a certain number of cigarettes. Groups so defined are pure from the conceptual points of view but may on the other hand contain small numbers of individuals. Discordance can also be defined as quantitative intra pair difference between two smokers. A twin pair where the one member smokes just few cigarettes a day and the other 20 cigarettes or more a day is no doubt discordant. The inclusion of such pairs will increase the number of pairs under study. Exposure time expressed as age at start of smoking could also be taken into consideration. An index of lifetime exposure expressed as number of cigarette years could be developed (cf section 4 3 2). The same principle may be used in forming discordance groups of nondrinkers versus drinkers or quantitative discordance could be measured in terms of absolute alcohol intake as expressed by milliliter of alcohol per month or per year. Few variables however except the two mentioned above lend themselves to quantitative assessment. Physical activity for example is basically of the ordinal scale type but may have to be treated as dichotomous for practical purposes.

If covariables which have an independent influence on the medical end point studied are assumed to be present the discordance groups should if numbers allow be selected from pairs who are concordant with regard to the covariable in question. This principle is termed restriction. For example the strong correlation between smoking and alcohol drinking should call for study of one discordant variable at a time while the other is kept constant. This situation could be ideal but is seldom met in practice. It is possible however to evaluate to what extent smoking discordant pairs are concordant or discordant in regard to drinking and on the basis of this information to assess the possible bias of the confounding.

If possible internal validity tests of factor discordance should be performed. In the present study response factors known to be smoking-related smoker cough was used to evaluate whether measurable difference existed in the strength of discordance between monozygotic and dizygotic pairs (cf section 7 2).

4 3 1 External environmental data

In the first Swedish questionnaire study questions were asked about the subjects lifetime residential histories permitting a rough classification of the respondents according to whether they had resided most of their life in urban or rural areas. The categories afforded were lived almost entirely in large towns, lived almost entirely in small towns or almost as many years in urban as in rural areas and lived almost entirely in rural areas.

The meaning of this urban-rural classification cannot be expressed in very clear terms. It may to some degree reflect an exposure to environmental air pollution but it may just as well reflect life-style parameters associated with urban or rural social environments.

A different approach was taken in the study on the US twins. Each respondent was asked to fill out a table stating the number of years after 1945 that he had spent at different locations within the US. At the same time the respondent was asked whether he was living and working respectively in a downtown, suburban or rural area. The information was used to set up different kinds of urban-rural exposure scores of which some utilised only the information contained in the individual's report as to type of area. The details of the computation procedure have been described (Brubec et al 1973). The indices were also used to establish discordance groups. In the most recent Swedish questionnaire study the same type of questions about lifetime residential history were used. This information has not been utilized for any analysis thus far.

4 3 2 Habitual data

Smoking habits

The main smoking classification in the twin research program in Sweden was based on questions asked in the very first questionnaire survey. The subjects were asked whether they were smokers or nonsmokers, about the type of smoking and whether their cigarette consumption was 1, 10, 11, 20 or more than 20 cigarettes per day. The classification of discordance in regard to smoking was performed according to the following principles.

Two groups were formed. One group contained nonsmokers versus cigarette smokers regardless of other type of smoking. The other group contained pairs of twins where both members were cigarette smokers but with differences in regard to the amount of cigarettes smoked. The low groups were "cigarette only" smokers while in the higher groups cigar or pipe smokers were also allowed. The contrasts as to cigarette smoking per day were the following:

Low smokers	--- High smokers
<10 cigarettes	--- >10 cigarette s
<20 cigarettes	--- >20 cigarettes

In many analyses the groups of nonsmokers versus cigarette smokers are pooled with groups of low smokers versus high smokers. These pooled groups are designated as pooled low group and pooled high group.

Although slight differences in the phrasing of the questions exist between the first Swedish questionnaire and the following questionnaire studies in Sweden as well as in the US the principles of forming smoking discordance groups have been the same.

It should be stated here that in some tabulations the discordance groups current and former smokers have been taken together while in other subgroupings only current smokers or former smokers have been presented. Which principle has been followed will be stated clearly in the result sections of this report.

In the clinical study by Lundman (1966) a different concept of smoking discordance was used. On the basis of more detailed exploration of the subjects' smoking habits during the years Lundman calculated a cigarette exposure index which he named number of cigarette years. The cigarette consumption of the subject was measured as the lifetime exposure which is the product of the mean number of cigarettes smoked per day and the number of years the subject had been smoking. The classification of discordance was then based on the intrapair difference giving quantitative measure of discordance.

Drinking

In the questionnaire surveys questions on drinking habits were introduced for the first time in 1967. Information about present habits was classified for each type of alcoholic beverage: beer, wine and strong liquor. The subjects were asked to state how often they indulged in drinking these kinds of beverages and how much they drank each time.

In addition to these questions the respondents were asked whether they often, sometimes or never on any occasion drank as much as half a bottle of strong liquor or one bottle of wine or in the American study four quarts of beer. This question led to dichotomy of the subjects into two classes: namely those who had answered never and those who admitted that they often or sometimes had drunk this much on any occasion.

In the clinical study by Myrberg (1974) alcohol discordance was evaluated in more detail on the basis of grams of alcohol per year. In his interview of the twin subjects Myrberg also identified certain behaviors related to high consumption such as blackouts, use of eyeopeners, drinking behavior, parties, intoxication, hangovers, as well as the above mentioned questions about drinking certain quantity on any occasion.

Another variable of considerable interest for the classification of alcohol behavior could be measured in the Swedish twin sample. In Sweden all persons who have been in conflict with the police in connection with alcohol abuse such as public misdemeanors, drunken driving or bootlegging are registered by the authorities. These records were screened for identical twins belonging to the registry. Accordingly the twins could be classified as "registered" or "nonregistered" and the pairs concordant or discordant. The biological implication of registration cannot be straightforwardly evaluated. The variable was shown to be clearly related to other measures of excessive alcohol consumption but it must also be assumed that the variable is not a direct measure of

1) This is not true if the discordance classification in mortality study on US twins (cf. section 8.3.4).

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Low smokers	---	High smokers
<10 cigarette	---	>10 cigarettes
≥20 cigarette	---	>20 cigarettes

In many analyses the groups of nonsmokers versus cigarette smoker are pooled with groups of low smokers versus high smokers. These pooled groups are designated as pooled low group and pooled high group.

was sought as to the use of tonics and vitamins prescription-free analgesics sleeping pills tranquillizer and oral contraceptives for women. In the dichotomy of these variables the cutoff point was placed between regular use during longer or shorter periods versus now and then and never.

alcohol consumption but certainly of a life-style parameter (Tibblin 1972) It should be kept in mind that the variable may be closely related to social class and that negatives by no means can be regarded as nonalcoholics

Physical activity

In the questionnaire of the old Swedish Registry the different response categories as to amount of physical exercise were respectively no exercise some exercise regular exercise and hard physical training A dichotomy was employed with the cutoff point between some exercise and regular exercise In the US questionnaire the questions specified physical exercise (outside of work) after 35 years of age Persons responding "hardly any or light exercise if a regular walks light gardening" were classified into one group and those responding that they participated in minor sports (swimming tennis etc) or hard physical training were classified into the other group

The questionnaire mailed to the new Swedish twin registry asked about exercise both at work and during leisure time The physical activity at work was judged according to four categories namely "mainly sedentary work work that to a large extent involves standing and walking but no other physical activity" work that involves standing and walking but also lifting and carrying" and heavy manual labor The cutoff point was placed between "mainly sedentary work and the other categories

The activity scale in regard to leisure time was based on a series of questions ranging from hardly any exercise to very hard exercise The cutoff point was placed between not very much exercise and rather much exercise

Change of employer

In both the Swedish and the US questionnaire studies questions were asked about how many times the respondent had changed employer since aged 25 Those in Sweden answering 3 or more times were classified into one group and those with few changes into another group

Food habits

Information on food habits was sought in both the Swedish and the US studies In the present report only 2 items connected to food habits have been included as they are supposed to be related to a life-style pattern These items are cooked food once a day or less and more than 5 cups of coffee a day

Several attempts were made to classify the subjects not so much according to total food intake in terms of calories as according to type of food yielding such groupings as predominantly vegetable and fruit eaters fish eaters meat eaters etc as such classifications were assumed to relate to smoking habits

Drug consumption

Consumption of drugs was investigated only in the new Swedish twin registry and in some subsamples from the general population Information

- 4 Are you sometimes happy or sometimes sad without any special reason?
- 5 Do you prefer to keep to the background in the company of other people?
- 6 Do you regard yourself as happy and carefree?
- 7 Do you often reach decisions too late?
- 8 Do you often feel tired and restless without any special reason?
- 9 Do you have a lively manner?
- 10 Can you quickly describe your thoughts in words?
- 11 Are you often lost in your thoughts?
- 12 Do you have anything against doing things or asking people for money for some charitable purpose?
- 13 Are you extremely sensitive in any respect?
- 14 Are you ever too restless to sit still?
- 15 Do you keep things to yourself except when in the company of good friends?
- 16 Do you have any nervous problems?
- 17 Do you like to crack jokes and tell funny stories to your friends?
- 18 Do you usually worry a long time after a distressing incident?

The items within each dimension were scored whereafter the individual mean score was classified as positive or negative in relation to the logical midpoint of nine-category scales.

Another indication of psycho-social disharmony was obtained from question appearing in the 1st Swedish questionnaire study. Individuals who considered their lives to be very stressful were regarded as positive.

In the Swedish questionnaire study of 1967/70 and partly in the US study a series of questions was asked in regard to the subject's adjustment to everyday life and occupation. The different items were presented in the form of statements to which the respondent could express a firm agreement or disagreement or one of three intermediate scale values designating partial agreement, uncertainty as to level of agreement and partial disagreement. The statements were the following:

- 1 I think that money has almost always been a problem for me
- 2 Financially I have not achieved what I have hoped for
- 3 I have often been annoyed by my inability to set aside work
- 4 My position has involved too much responsibility
- 5 My training has not been adequate for the work I am doing
- 6 I have achieved the position (within my vocation) to which I have aspired
- 7 My ability and training have been used to the full
- 8 I have had a lot of difficulty in my marriage
- 9 My children have been a big problem for me
- 10 I have always gotten along very well with my parents
- 11 I have always gotten along very well with other people
- 12 For the most part I have gotten along well with my coworkers
- 13 I have often run short of time
- 14 I have often had difficulty in finding enough time to complete the work assigned to me
- 15 I have often been restless
- 16 I have often had difficulties in falling asleep
- 17 I have often felt somewhat uneasy in my work

Floderus (1974) grouped the items as being reflections of financial problems (1-2), occupational maladjustment (3-7), interpersonal conflicts (8-12), shortage of time (13-14) and unspecific maladjustment (15-17).

In the US study an index of the occupational maladjustment was calculated in accordance with principles developed by Likert (see e.g. Edwards 1957) to form a psycho-social score based on items 2, 4, 7, 12, 14 and 17.

In the last Swedish questionnaire study on the group of twins born between 1926 and 1938 the psycho-social status of the individuals was measured in a more specific way. On the basis of the principles developed by Eysenck and Eysenck (1964) in their psychological inventory scale a modified scale was designed for questionnaire use. This scale was assumed to measure two psychological dimensions, namely stability and extravertness. The details of the procedure in developing the scale have been reported by Floderus (1974).

The subject was instructed to answer yes or no to the following questions

- 1 Do you like having a lot of things going on around you?
- 2 Are you often uneasy feeling that there is something you want without knowing what it is?
- 3 Do you almost always have an answer ready when spoken to?

- 4 Are you sometimes happy or sometimes sad without any special reason?
- 5 Do you prefer to keep to the background in the company of other people?
- 6 Do you regard yourself as happy and carefree?
- 7 Do you often reach decisions too late?
- 8 Do you often feel tired and restless without any special reason?
- 9 Do you have lively manner?
- 10 Can you quickly describe your thoughts in words?
- 11 Are you often lost in your thoughts?
- 12 Do you have anything against selling things or asking people for money for some charitable purpose?
- 13 Are you extremely sensitive in any respect?
- 14 Are you ever too restless to sit still?
- 15 Do you keep things to yourself except when in the company of good friends?
- 16 Do you have any nervous problems?
- 17 Do you like to crack jokes and tell funny stories to your friends?
- 18 Do you usually worry a long time after a distressing incident?

The items within each dimension were scored whereafter the individual mean score was classified as positive or negative in relation to the logical midpoint of nine-category scale.

Another indication of psycho-social disharmony was obtained from question appearing in the last Swedish questionnaire study. Individuals who considered their lives to be very stressful were regarded as positive.

4 5 Classification of Medical Endpoints

4 5 1 Data from the questionnaire surveys

All Swedish questionnaire surveys except the very first one contained questions on the medical status of the individual. Of greatest importance were some questions pertaining to cough and coronary heart disease. The questions on cough were modified from a similar questionnaire¹ worked out by the College of General Practitioners of the British Medical Research Council (1960). The questions in the twin studies were the following:

- 1 Do you have a cough regularly or for extended periods of time?
- 2 For how many months in a row do you cough per year more or less than three months in a row?
- 3 For how many months in a row do you cough up phlegm from your chest more or less than 3 months in a row?

The subjects were classified as having cough² if the answer to the first question was affirmative; as having prolonged cough if the answer to the second question indicated cough for more than 3 months in a row; as having "bronchitis" if the answer to the third question divulged that they coughed up phlegm for more than 3 months in a row.

The question on heart symptoms focused on the possibility of diagnosing angina pectoris. The questions were somewhat modified but mainly based on the questionnaire worked out by Rose (1962) at the London School of Hygiene and Tropical Medicine and ran as follows:

- 1 Have you ever had any pain or discomfort in your chest?
 - (a) No
 - (b) Yes
- 2 When do you feel this pain or discomfort?
 - (a) When you are emotionally upset or excited
 - (b) When you walk fast or walk uphill
 - (c) When you walk at normal speed on level ground
 - (d) Under other circumstances
- 3 What do you do when you feel the pain or fort while you are walking?
 - (a) Stop walking or walk more slowly
 - (b) Take medicine and continue walking at the same speed
 - (c) Continue walking at the same speed without taking medicine
- 4 If you stop walking regardless of whether you take medicine or not how is the pain or discomfort then?
 - (a) The pain usually subsides within 10 minutes
 - (b) The pain usually continues for more than 10 minutes

1) The phrasing was slightly different in the US study
 2) Symptom in quotations designates questionnaire diagnosis

5. Where is the pain or discomfort located?
- () In the middle of the chest
 - (b) In the left side of the chest
 - () In the left arm
 - (d) In some other place

Two slightly different criteria were used for diagnosing angina pectoris in regard to the answer to question 2. Common to both of the criteria was that the answer to question 1 should be affirmative; question 3 should be answered with a and not b question 5 with a B or but not d. For diagnosis of angina pectoris used solely by Liljebo (1970) it was required that question 2 should be answered by checking either b or c and not d. In the less restricted diagnosis of angina pectoris used in most reports alternative was regarded as sufficient for making the answer under heading 2 affirmative.

Apart from the above-mentioned symptoms the questionnaire employed in the New Swedish Twin Registry asked for information about symptoms of shortness of breath, touch and back disorders, severe headache, hearing loss and allergic predisposition.

As for diseases the 1963 Swedish questionnaire asked for information about present illness or illness occurring during a period of 5 years prior to the questionnaire. Information was also sought as to whether the disease had necessitated an examination by a doctor or hospitalization. Among diseases included in this list were asthma, bronchial catarrh, heart attack, angina, high blood pressure, gastric ulcer, nervous diseases and diabetes. The questionnaire in Sweden 1967/1970 and the US questionnaire also asked about diseases such as asthma and allergic manifestations.

The questionnaire employed in Sweden in 1973 did not give a list of earlier diseases but asked whether the subjects were suffering or had suffered any long lasting or serious diseases or whether he had leave of absence on the grounds of any disease for any considerable length of time.

4.5.2 Data from the medical examinations

The clinical examiners used variety of tests available to them at the well-equipped hospital including radiological examinations of heart and lung function tests like respirometry and exercise electrocardiogram and variety of blood and urine examinations. The methods and procedures are described in detail in their reports and will not be taken up in the present one. The methods will be properly referred to later in conjunction with the presentation of the results.

4.5.3 Gross and cause-specific mortality

On all cases in the first Swedish questionnaire survey of 1961 mortality has been followed up during the ensuing years. Matching has been done at regular intervals with death certificates kept by the Central Bureau of Statistics. Besides the underlying and contributory causes of death the certificate which is signed by physician gives information on whether the patient had been treated in hospital. Hospital records information from general practitioners and other pertinent information were collected to the extent that they were available. The cause of death was

then established in the following way: Without knowledge of important covariables such as smoking and drinking habits one physician (de Faire) studied available records and made up a preliminary diagnosis of the cause of death. A final evaluation was thereafter made by him and two of the authors (LF and TL) whereby the causes of death was classified according to the WHO International Statistical Classification (ISC) of Diseases, Injuries and Causes of Death (1967). Finally it was assigned to one of the following groups:

<u>Cause of death</u>	<u>ISC number</u>
Coronary heart disease	410-414 795
Cerebrovascular disease	430-438
Cancer of the lung	162
Cancer other forms	140-161 163-239
Suicides	E950-E959
Accidents	N800-N999 E800-E949
	E960-E999
Other causes	—

4.6 Validity Aspects

4.6.1 Validity of measurements

Data from questionnaire studies are subjective. The validity of subjective measurements stand and falls with the truth of the information given by the individuals themselves. Several sources of error may operate of which some are related to the questionnaire and the phrasing of the questions and other to the interplay between the interviewee and the interviewer. Perhaps most important are errors that may be inherent to the interviewee himself. For retrospective assessment of exposure memory may play a significant role but the greatest difficulties seem to be associated with the subject's own assessment of what exposure category he may belong to. His manner of giving information about himself may also be influenced by preconceived ideas. There is often a tendency on the part of the interviewee to make himself better in the eyes of the investigator (Cederlöf Friberg and Jonsson 1963; Selitis et al 1966).

For example, in regard to external environmental data such as urban-rural residence history, the memory of say a 60 year-old subject may not be good enough for him to state the year precisely. He may also have looked shorter periods of urban residence which were indeed of no significance to himself but which might have been important for the investigator to give him proper rating with regard to possible air pollution exposure. Even more important than the subject's memory or value judgements may be his inner uncertainty as to whether his residential area is classifiable as urban, suburban or rural.

Validity checks in regard to the subject's actual migration pattern could possibly be made in Sweden on the basis of migration records but this procedure would have involved an enormous amount of work. It therefore has to be accepted that the report on the residential history may not be too strong a measure in the individual case but that it at least may afford the possibility of making a dichotomous assessment.

The accuracy of the exposure index developed on the basis of air pollution measurements estimates in the DS study can also be questioned. Again there is no easy way to perform validity evaluation. As correlations between air pollution indices and medical endpoints were rarely found, the validity of the classification may have been weak.

As for habitual factors such as smoking and drinking, the respondent may have forgotten temporary use of cigarettes or alcohol many years ago. The use of classification brackets like 1-10 cigarettes or 11-20 cigarettes in the first Swedish questionnaire study presupposes an average estimate on the part of the respondent that may not be clear to him. The respondent may tend to underestimate how much he has smoked as well as how much he has consumed alcoholic beverages. This underreporting may be subconscious and is likely to occur more often in regard to habits that are socially questionable such as excessive drinking and drug consumption than in regard to socially accepted habits such as smoking.

The only entirely satisfactory way to validate the questionnaire response would be to perform some kind of observational studies during the same

then established in the following way. Without knowledge of important covariables such as smoking and drinking habits, one physician (de Faire) studied available records and made up a preliminary diagnosis of the cause of death. A final evaluation was thereafter made by him and two of the authors (LF and TL), whereby the causes of death was classified according to the WHO International Statistical Classification (ISC) of Diseases, Injuries and Causes of Death (1967). Finally it was assigned to one of the following groups:

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Suicides	E950-E959
Accidents	N800-N999 E800-E949
	E960 E999
Other causes	—

Some validity analyses were performed in the Lundman (1966) and Lilje-
fors (1970) clinical studies to measure the reliability of the question-
naire diagnosis of cough "bronchitis and angina pectoris". The
physician (Lundman) reinterviewed the subject in regard to bronchiti-
s and dynamic spirometry was performed to ascertain airway resistance.
Nitrogen delay washout was determined by multiple breath method in
order to detect uneven ventilation.

Out of 23 cases that were classified as having "bronchitis" according to
the questionnaire 9 or less than half were so classified according to
clinical anamnestical criteria. Out of 343 cases that did not have bron-
chitis according to the questionnaire 7 or two percent were posi-
tives. This outcome is not unexpected as the criterion for being posi-
tive is rather difficult to define while the criterion for being
negative is easy to comprehend. The relation between the "bronchitis"
report on the questionnaire and the lung function test (MCO) showed that
seven of the 23 "bronchitis" cases had a decreased lung function. In
this evaluation however 53 cases among the 339 nonbronchitis sub-
jects or 16 percent showed an impaired lung function (Lundman 1966).

An indirect way of measuring the relevance of a symptom is to find out
to what extent it is related to a confirmed causal factor. Though logi-
cally appealing it should be emphasized that if the disease endpoint is
only assumed to be related to the causal factor the confirmation process
is not a scientific way of validation. As smoking is known to relate
causally to certain respiratory symptoms an analysis of this kind was
performed on data from the twin study. The results showed a loose rela-
tion between the respiratory symptoms and breakdown in regard to
smoking habits (of section 7.2).

An attempt to validate the diagnosis angina pectoris was also made on
empirical data. Earlier literature had revealed that this symptom
diagnosed by questionnaire administered in personal interviews had
good prognostic value (Rose 1962; Rose 1964). However, as the present
investigations employed mailed questionnaire methods the questions had
to be somewhat modified and shortened in such a way that might alter the
validity. In publication by Cederlöf Jonsson and Lundman (1966)
based on questionnaire data as well as on the clinical examination of
smoking discordant pairs by Lundman (1966) it appeared that out of 8
mailed patients with angina pectoris according to the questionnaire 4
were confirmed to manifest cases of coronary heart disease. Among 204
mailed cases not displaying the symptom according to the questionnaire
2 (1 percent) were clinically positive. The validity was poor for women.
A sample consisting of 69 mailed twins from the combined Lundman and
Liljefors clinical studies (Lundman et al 1971) who had been diagnosed
according to the questionnaire having angina pectoris were subse-
quently clinically examined in regard to medical history, physical signs
and exercise electrocardiogram. The clinical diagnosis of angina pecto-
ris was based on the criteria recommended by the WHO expert committee on
arterial hypertension and ischemic heart disease (WHO 1963). The ECG
test was considered pathological according to the principles in the
Minnesota code (Blackburn et al 1960). Lundman et al (1971) reported
that depending on what criterion was used in diagnosing angina pecto-
ris on the questionnaire (section 4.5.1) the confirmation rate based
on the anamnestical finding at the clinic was 22 percent or 33 percent.
The last figure referring to the more restrictive criterion. When the
investigators added other signs of coronary heart disease like overt

period of time as the questionnaire was being sent out. Personal interviews can hardly be regarded as more reliable; on the contrary the underreporting may be even more pronounced in a personal interview where the subject has to face the interviewer than in a questionnaire. This consideration notwithstanding some comparisons which have been made between 140 subjects' responses on the 1973 Swedish questionnaire and a subsequent personal interview with these same subjects (Medlund et al. 1977) may be of interest.

In regard to the qualitative assessment of smoking - i.e. whether the subject was a nonsmoker, a former or a present smoker - 132 out of 140 subjects or 94 percent gave the same response on the interview as they did on the questionnaire; in only one case could the deviating answer at the time of the interview not be explained by possible changes between the two occasions. One indication of the possible extent of underreporting is found in a study by the Swedish Central Bureau of Statistics (1965). Based on questionnaire responses in a sample of 55 000 Swedish men and women, the total consumption of tobacco was estimated to be 89 percent of the tobacco goods actually sold during the period in question. However, this finding could just as well be explained by nonresponse as by underreporting.

As to alcohol consumption the quantities reported at the time of the interview consistently tended to be lower than what was stated on the questionnaire. Björkman (1971) emphasizes that such discrepancies are most pronounced in regard to excessive consumption and that the mailed questionnaire approach seems to give more valid estimates than a personal interview study.

In regard to the covariables used in the twin studies, comparisons between questionnaire and interview reports were made only to a limited extent. In this context a shortened version of the questions about physical activity correlated well ($r=0.87$) with an index based on a detailed description of type of activity and degree of exertion. Further answers to questions about food habits agreed more than 90 percent between questionnaire and interview. The measures of psycho-social instability developed by Floderus (1974) were correlated to consumption of psychotherapeutic drugs. It was found that the instability groups showed a strikingly frequent intake of such drugs, i.e. up to four times the intake reported by stable subjects. This was assessed as pointing to a high degree of classification validity.

Experience in using questionnaires for medical diagnoses is not very extensive. In most studies reported, the subject had answered the questionnaire in connection with a personal visit to the hospital or clinic. The medical personnel then have a chance to explain difficult questions and to complete questions which the subject on the first go-around neglects to answer.

The main difficulty with mailed questionnaires may well be that the subject has to classify himself into a series of categories which he may find imprecise and incongruent with his own sense of reality. Granted it is possible to get the respondent to go back and fill in parts that he has missed by mailing the questionnaire to him a second time; nonetheless there is no way of explaining the phrasings of the questions. On the other hand it is unlikely that the respondent has major reasons for distorting his answers to medical questions as he may have in regard to habitual variables.

To what extent the twin series in the present program represents the general population depends on several factors. One factor relates to possible biological, environmental and social differences between twins and non-twins. Another factor is the very restriction that both twins should be alive as an unbroken pair at the time of their inclusion into the study. Other questions concern the adequacy of subgroupings and rate of nonresponse.

The question of differences between twins and singletons has been thoroughly discussed by one of the authors (Cederlöf 1966) on the basis of comparisons between the twin series and sample of about 900 singletons in the same age bracket. The twin series used for the epidemiological analysis was found to be highly selected with regard to age and sex. This fact stems from among other things differences in mortality rates between twins and singletons (Esson-Möller 1941). An unpredictable bias with regard to health status is thus engendered among the couples in which both partners have survived. This implies that the calculated prevalence rates even if sex and age-standardized should not be regarded as unbiased estimates of any population parameters. On the other hand the empirical comparisons with regard not only to medical data but also to some environmental and sociological background variables did not reveal any large consistent differences between twins and singletons. However, strongly the lack of representativeness in regard to age and sex deserves to be emphasized. The remarkable conformity between twins and singletons with regard to the variables under study speaks in favor of broader generalization than could have been expected a priori. The twin material although not representative for the age and sex structure of the population stands in definite relationship to the defined population of couples surviving pairs and is emphatically not selected series.

Of major importance is the validity of the subgroupings of the total material both in regard to zygosity and to the focus-variable of the study, smoking. The validity of the zygosity diagnosis has been discussed in another context (cf section 3.3). The comparability between the monozygotic and dizygotic series in regard to exposure discordance is crucial. However, and efforts have been made to study to what extent the smoking discordance is the same in the two zygosity groups. There are several sets of data that can be used for such an evaluation.

In the paper by Friberg et al (1973) the question of comparability between monozygotes and dizygotes in regard to smoking discordance were discussed in detail based on data from the 1961 questionnaire as well as the 1967/70 questionnaires. The comparability of the groups appears in table 4.1, page 50 which is extracted from the data presented. There are no indications of relevant difference between monozygotes and dizygotes in any of the age- and sex-groups. The time of exposure was not evaluated, however, and some data from Lundman (1966) might indicate the existence of slight difference in total exposure. In his sample of smoking discordant pairs mean intra-pair difference of 259 cigarette year (cf section 4.3.2) for 77 monozygotic pairs as against 316 cigarette year among 89 dizygotic pairs.

coronary heart disease suspect angina pectoris and pathological ECG the confirmation rates were 57 and 62 percent respectively. There is all reason to believe that these figures overestimate the validity as part of the subjects clinically examined were screened not only by the questionnaire but also by a telephone interview which was not discussed in the report.

Another way to elucidate the validity of the symptom is to estimate its prognostic value. This was analyzed in the group consisting of all 256 twin pairs where the one partner was diagnosed as having angina pectoris according to the questionnaire while the other partner did not. The relative risk of death regardless of cause was 1.8 (40/22 deaths); if only deaths from coronary heart disease were considered the relative risk was 2.5 (15/6 deaths).

In summary it can be concluded that the questionnaire is useful for screening cases with CHD as the sensitivity is quite high. If it is used for prevalence studies or effect studies one has to be aware of a high frequency of false positives.

An elucidation of the validity of some other symptoms can be taken from the New Swedish Twin Registry where all the questions on symptoms (section 4.5.1) on the 1973 questionnaire were asked in a subsequent interview on a subsample of 140 individuals. The agreement was consistent ly between 90 and 100 percent.

Gross mortality has an absolute validity provided that all causes of death are reported. With some highly insignificant exceptions this is true for Sweden. Since the registry has operated with annual follow-ups of mortality no recorded cases of deaths have escaped notification.

As for specific cause of death however there is still room for some inaccuracy. In order to study this an investigation was made on the 1,290 twins that had died up to 1973 in the age groups of twins born 1901-1925 (de Faire et al. 1976). Apart from the official death records for most cases information was available from hospital records often including autopsies or at least from local physicians giving a total number of 1,156 deaths to be evaluated. The specific cause of death was assessed based on the official death certificate only as well as based on the compiled information only. The statistical treatment of the outcome was expressed in terms of sensitivity i.e. to what degree the death certificate disclosed the true cause of death according to the compiled information. The other measure was the so-called rate of confirmation which starting from the death certificate expressed how reliable the diagnosis is in comparison with the true cause. For most diagnoses both sensitivity and rate of confirmation approached 95-100 percent. In regard to sensitivity some noteworthy exception should be pointed out. Among all cases of leukemia only 81 percent were recorded on the death certificate; the corresponding rate was for diabetes mellitus 60 percent and for alcoholic psychosis, alcoholism and drug dependence 81 percent. The conclusion stated by the authors was that Swedish death certificate data are fairly valid for use in epidemiological studies and mortality statistics with regard to most cancers, cerebrovascular disease, ischemic heart disease, bronchitis, asthma and emphysema, accidents and suicides but not for diabetes mellitus, alcoholism, mental disease, rheumatic heart diseases and other heart diseases. However, in selected clinical-epidemiological studies all available documents should be collected in every case prior to judging the cause of death.

4 6 2 Validity of sample and subgroupings

To what extent the twin series in the present program represents the general population depends on several factors. One factor relates to possible biological, environmental and social differences between twins and non-twins. Another factor is the very restriction that both twins should be alive as an unbroken pair at the time of their induction into the study. Other questions concern the adequacy of subgroupings and rate of nonresponse.

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Table 4.1 Cigarette Exposure 1967/1970 by Sex Age and Zygosity in Smoking Discordant Twin Pairs According to the 1961 Questionnaire Study (Part of B series All pairs that answered the questionnaire in 1967/1970)

Nonsmokers versus smokers	Born	DIZYGOTES				MONOZYGOTES			
		No of Non- pairs smokers		Smokers Difference		No of Non- pairs smokers		Smokers Difference	
Males	1911-25	278	0 6	+6 4	7 0	89	0 9	+7 0	
	1901-10	120	0 2	+5 4	5 6	36	0 3	+5 5	
Females	1911-25	429	0 3	+6 7	7 0	191	0 5	+6 0	
	1901-10	134	0 1	+6 0	6 1	47	0 3	+4 7	
Pooled low versus pooled high group		No of pairs		Low		High		No of pairs	
Males	1911-25	389	2 3	+6 8	9 1	140	3 6	+6 4	10
	1901-10	156	1 6	+5 4	7 0	50	1 8	+5 2	7
Females	1911-25	495	1 4	+6 3	7 7	231	1 7	+5 6	7
	1901-10	151	0 9	+5 7	6 6	51	1 0	+4 3	5

Another approach to evaluate possible differences between the zygosity groups was to use the relation between smoking and the questionnaire symptoms cough and prolonged cough (cf table 7.2). It can be seen from the table that there is no general trend that the monozygotic pairs should be less discordant than the dizygotic.

Possible error of measurement in regard to the smoking habit may affect the composition of the subgroups concordant nonsmokers discordant smokers and concordant smokers. Such possible errors have a greater effect on the discordance group which will contain a certain number of concordant nonsmokers and smokers who have reported themselves as discordant (Friedman 1977). The effect of this error will be reflected in lower relative risks in the discordance group than what would be expected on the basis of the A series analysis. The size of this deflation of the risk ratio could be theoretically estimated but so many assumptions are involved that it has been considered hardly justified. There is no reason to believe that any error for the responses on smoking is different between the monozygotic and dizygotic series. If a difference should exist it seems more reasonable to assume that the monozygotes would more often tend to give a too concordant report than would the dizygotes. In fact as will be later shown (section 7.3) the risk ratio in regard to mortality in the dizygotic series is not far from what can be expected on the basis of the A-series analysis.

The importance of a high response rate for an epidemiologically valid assessment of results has been taken up by several authors (Kroege et al 1970; Kaplan and Col 1970; Elli Edo and Arner 1970). The nonresponse rate is calculated as the number of twin pairs where non or only one of the partners returned his questionnaire in relation to the total sample of located pairs approaches 20-25 percent in some of the studies. The nonresponse rate is not higher than reported in some of the studies using mailed health questionnaires where for example Dorn had a non-response rate of 32 percent (Kahn 1966). It has been shown (Seltze Boese and Garvey 1975) that nonsmokers tend to be more willing to return their questionnaires than smokers or measured as response within six months almost 100 percent as against 92.94 percent. This tendency was also shown to be related to amount smoked among present cigarette smokers in such a way that the heavier smokers were less willing to return the questionnaire promptly.

HEREDITARY ASPECTS OF MEDICAL ENDPOINTS AND BIOLOGICAL RISK FACTORS

Introduction

Some leads for the epidemiological analysis of the possible health effects of smoking may be obtained by investigating to what extent a certain medical endpoint has a higher probability of occurring in the co-twin of a proband who is trait positive as compared to a co-twin of a proband who is trait negative. The present paper makes an attempt to study these relations on the basis of the method of analysis generally employed to test the importance of genetic factors in contrast to environmental factors, namely the classical twin method" (cf section 2.1.2).

A brief overview will now be given of the present attitude towards the influence of genetic factors on obstructive lung disease, lung cancer and ischemic heart disease as well as on some biological risk factors.

For many years chronic obstructive lung disease and especially chronic bronchitis was believed to be entirely secondary to environmental determinants. This is understandable since air pollutants such as cigarette smoking, dusty factories and cities and lung irritants such as gases have been shown to have strong deleterious effects on the lung function. Repeated infections by bacteria, virus, mycoplasmas and fungi are also considered as environmental factors, the negative effect of which cannot be denied (Leeder 1975). However, during the last few years data has appeared showing that these environmental factors exert a powerful effect on the lung function and structure if some prerequisites of possible genetic origin are met (Riggins and Keller 1975; Larson et al 1970). These factors include airway obstruction, lack of immune defence, deficiency of inhibitors of proteolytic enzymes and depressed ciliary function.

Individuals with some degree of airway obstruction early in childhood, especially those with asthma, are prone to repeated infections and the subsequent development of chronic bronchitis. Strong evidence has been put forth to the effect that asthma and allergic rhinitis occur in families and that they are inherited in some way (Schwartz 1952; Leigh and Marley 1967).

The immune defence is of paramount importance for protection against respiratory infection. Persons with some immunoglobulin deficiencies exhibit recurrent upper respiratory tract infections. Selective immunoglobulin A (IgA) deficiency is the most common occurring in one out of 500 to 700 persons (Johansson, Högman and Killander 1958; Bachman 1965). In large families where several members either were deficient or had borderline values of IgA, Webb and Condemi (1974) found a relation between the deficiency state and chronic bronchitis and emphysema. They suggested an intermediate inheritance of the trait.

Leurell and Eriksson (1963) and Eriksson (1963) were first to describe the association between α_1 -antitrypsin deficiency and emphysema. Numerous reports have later confirmed this association and the hereditary nature of the trait (Haas 1974; Lomström et al 1975; Winzler

Braun and Grob 1974; Lieberman 1969) The theory is that several proteolytic enzymes in the absence of this inhibitor destroy the lung parenchyma thus producing bronchiectasis and emphysema (Lieberman 1973) These changes favor new infections and inflammations which are the main causes of protease-production The evidence that the homozygotes of alpha₁-antitrypsin deficiency have high incidences of chronic bronchitis and emphysema is now very strong It may also be that the heterozygotes with intermediate alpha₁-antitrypsin deficiency are predisposed to chronic obstructive lung disease even if this question is still controversial (Mittman Lieberman and Rumsfeld 1974; Cole et al 1976; Cooper et al 1974) The heterozygotes may constitute up to 14 percent of the population

The pathogenesis of chronic bronchitis is thus dependent not only on environmental factors but also on genetic ones In fact there is an important interplay between the inherited traits and the environment

There are no conclusive data as to a genetic influence on the development of lung cancer though a tendency to aggregation of lung cancer in human families in the absence of smoking has been reported (Tokuhata 1964) During recent years data have accumulated which that genetic factors may operate in the development of lung cancer in smokers A summarizing discussion is put forth in the Surgeon General Report (1975) The possibility is pointed out that the enzyme aryl hydrocarbon hydroxylase (AHH) may be genetically determined and may mediate an increased susceptibility to certain carcinogens found in tobacco smoke One finding along this line is a higher prevalence of subjects homozygous for an allele able to induce high AHH levels among lung cancer patients compared to healthy controls and tumor controls (Kellermann Shaw and Leyten Kellerman 1973)

Recently Cederlöf and Friberg (to be published) observed two monozygotic pairs where both members had died from lung cancer In both pairs one member was classified as smoking 11-20 cigarettes while the other member was smoking cigar and pipe only Expected number of concordant pairs based on age and smoking group specific prevalence rates in the total group of 227 present smoking concordant monozygotes in these age groups was only 0.27 pairs which is significantly different from the observed value These data support a hypothesis that genetic factors may interact with smoking in the development of lung cancer Further it should be noted that two of the cases did not smoke cigarettes but cigar and pipe only

The genetic mechanism in coronary heart disease is of polygenic nature and is also mediated through most of the so-called biological factors For instance the serum cholesterol level can be controlled by several genes affecting the absorption synthesis transport and removal of cholesterol in the human body This polygenic control of biological factors must naturally result in a continuous distribution of such factors when measured in a population Some extremes of a factor e.g. the Fredrickson type II-A hyperlipoproteinemia may exhibit single gene control but in the same individual there must be other factors with are under the common polygenic influence (Fredrickson 1971)

Previous studies performed regarding heredity and coronary heart disease have primarily been concerned with familial aggregation of the disease but investigations dealing with blood pressure, glucose tolerance and serum lipids have also been made.

A familial aggregation of coronary heart disease has been found to exist (Gertler and White 1954; Thomas 1958; and Hammond, Garfinkel and Selman 1971). Russek and Lohman (1958) compared the family histories of 100 young coronary heart disease patients and those of 100 control patients. A definite history of cardiovascular disease in one or both parents was found in 67 percent of the patients with coronary heart disease. In only 16 percent were both parents living and enjoying normal health while in 17 percent one or both parents had died from noncardiac or unknown causes. In contrast, 40 percent of the control subjects had a similar history of cardiovascular disease in one or both parents while 40 percent indicated that both parents were living and enjoying normal health. Thomas (1958) obtained the history of the parents and grand parents of 724 medical students at the John Hopkins School of Medicine. It was found that cardiovascular disease was 2.7 times more frequent in offspring of affected parents. Retrospective studies of this kind can be criticized since persons with coronary heart disease are more likely to be aware of family members with similar symptoms than are unaffected persons.

In the prospective study of Hammond, Garfinkel and Selman (1971) the relation between coronary heart disease and longevity of parents and grandparents was investigated in 1,000,000 men and women. The persons in the study were traced 15 years after they had answered a detailed questionnaire. The subjects were divided into seven groups according to longevity of their parents and grandparents. 18.18% of the men and 7.07% of the women had died from coronary heart disease and a direct correlation was found between the mortality and the longevity-class of parents.

Twin studies using unselected larger samples concerning coronary heart disease are rather few. In the Danish twin registry (Harvald and Ruge 1970) 352 coronary deaths had occurred up to 1968. The individual concordance for the male monozygotic pairs 39 percent differed significantly from that of the male dizygotic pairs 26 percent. In the female pair the difference was greater 44 and 14 percent respectively. The risk of fatal coronary death was higher in the monozygotic pair than in the dizygotic pair. No such differences were found for the male pair. Harvald and Ruge concluded that the occurrence of fatal coronary occlusion seemed to be genetically determined to a much larger extent in females.

There is no doubt that type II-A hyperlipoproteinemia is of genetic origin (Fredrickson 1971) but the situation of the other types of serum lipids is less well known. Some important family studies have been performed on this subject. Nikkila and Aro (1973) have studied 412 first-degree relatives of 101 young survivors of myocardial infarction. They found that the mean levels of serum cholesterol and serum triglycerides were significantly higher than in control population but lower than in the index patients. Hypercholesterolemia occurred 1.8 times more frequently in relatives than in controls. Goldstein et al (1973) selected 164 hyperlipidemic infarct survivors and studied first second-

and third-degree relatives to these index cases. There was no correlation between husbands and wives speaking against a general environmental effect on lipid levels. The authors were able to disclose three types of familial hyperlipidemias, all of them associated with premature coronary heart disease.

From the twin studies performed by Osborne et al (1959), Meyer (1962) and Jensen et al (1965), evidence has been found for both the genetic and environmental influence of cholesterol and triglyceride levels. Twin pairs living together were found to have lower mean intrapair variance compared to those living apart.

Concerning other coronary heart disease risk factors such as high blood pressure and diabetes mellitus, investigations supporting a genetic influence also exist. In twin studies by Osborne et al (1959) and Takken (1964), the variability of both systolic and diastolic blood pressure was found to have a strong genetic influence. The same has been found regarding diabetes mellitus in studies by, for instance, Harvold and Borge (1965). In a study on monozygotic twins discordant with respect to overt diabetes mellitus, Cerasi and Luft (1967) found a closely similar insulin response after glucose infusion, indicating a genetic influence.

Respiratory Findings

The large scale questionnaire studies on the twins have contributed data on cough that have been used for calculating coincidence rates (Cade 1967 and 1967). In pair concordant for smoking the coincidence rates were consistently higher than expected and the coincidence was higher among monozygotic pairs than among dizygotic pairs.

A special analysis was performed on smoking-discordant monozygotic pairs divided into two groups according to the cough-status of the nonsmoking partner. On the basis of the conditional probability of "cough" occurring in concordantly nonsmoking pairs an expected number of coughers could be calculated for the smokers in the two above mentioned groups. The results appear in table 5.1.

Table 5.1 Expected and Observed Prevalence Rate (per cent) of Cough Among Smoking Partners to Co-twins Who Either Had or Had Not the Symptom "Cough" Monozygotic Pairs

Coughing status in non smoking partner	No at risk	Expected	Observed
No cough	497	4	12
Cough	41	24	37

Obviously the smoke had an increased risk with a factor of about 3 provided his co-twin was healthy. It that the smoke could be assumed not to have the predisposition for the symptom. However if he had this predisposition shown by the status of his nonsmoking partner the increased risk is about 1.5. Of further interest is to compare the observed to 12 percent among the smoking partners of healthy co-twins with the conditional probability of 24 percent in the group of cough among nonsmokers. The figure implies that "heredity" is more important than smoking for the development of the symptom. However the predisposed group is less than ten percent which will produce fewer coughers than does smoking. The observations were confirmed in the New Icon Twin Study (Cade 1967 Friberg and Hrubec 1969).

In the new Swedish twin registry the age conditional probability for cough among nonsmokers in both males and females was 17 percent in the monozygotic and 13 percent in the dizygotic series. No significant effect.

An indication of hereditary influence on respiratory function was provided by experiment on series of nine monozygotic and nine dizygotic twin pairs. Camille Philipeau and Friberg (1972) studied tracheo-bronchial clearance by external measurements of radioactive test aerosol containing monodisperse fluorinated ethylene propylene particles tagged with ^{99m}Tc . The clearance patterns were much more similar between the partner in the monozygotic pair than in the dizygotic pairs. These results concluded that the results indicate that tracheo-bronchial clearance is to great extent constitutionally determined.

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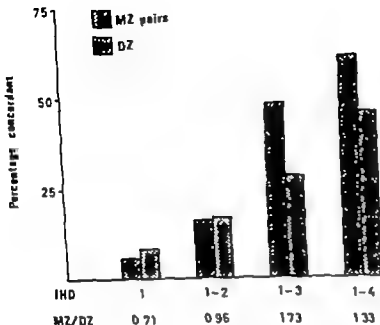


Figure 5.2 Cumulative concordance rates successively including IHD-groups 1-4; IHD-group 1 Myocardial infarction; 2 Angina pectoris ST depression during exercise; 3 Angina pectoris or ST-depression during exercise; 4 Findings of suspected IHD (From Liljefors 1970)

disygnoti pairs did not differ significantly indicating both environmental and genetic influence. In regard to triglycerides genetic factors seemed to have greater importance than environmental ones in females.

5.3.3 Mortality

The continuous mortality follow-up of the twin registry (cf section 4.3.3) has offered possibility of calculating the influence of genetic factors on gross and cause-specific mortality.

Up to December 1973 2760 deaths (1420 male and 1360 female) had occurred. 481 pairs were death concordant and 1818 death discordant. Death concordance rates (figure 5.3 page 60) were calculated for coronary heart disease, cerebrovascular disease and cancer (de Faire, Friberg and Lundean 1975). Male monozygotic twins revealed a significantly higher concordance rate for coronary heart disease than the male dizygotic twins (15.8 vs 8.0 percent). A similar trend was noted among the female twins but the difference (11.0 vs 7.5 percent) was not significant. With respect to death from cerebrovascular disease and cancer monozygotic and dizygotic twins displayed only slight differences.

5.3.1 Morbidity

Analyses on data from the older cohorts of the registry (Cederlöf Friberg and Jonsson 1967) revealed that with the exception of males aged 36-55 the coincidence rate of the symptom angina pectoris according to the questionnaire diagnosis was higher among monozygotic twins than among dizygotic ones. It was concluded that even though the questionnaire diagnosis angina pectoris to some extent includes cases with unspecific chest pain that cannot be verified clinically the results of the present investigation must be regarded as strongly supporting the idea of a genetic influence on the development of true angina pectoris.

In the clinical study by Lundman (1966) the observed coincidence rates for the clinical diagnosis coronary heart disease were 2.2 percent among 92 monozygotic pairs and 1.0 percent among 104 dizygotic pairs as against expected rates of 0.1 and 0.3 respectively. The results were based on small numbers however. Findings in regard to arcus lipoides corneae usually correlated with CHD revealed a highly significant coincidence of 21.7 percent among monozygotic twin pairs and 5.8 among dizygotes.

In Liljefors' (1970) clinical twin study the hereditary aspects of coronary heart disease were also evaluated. When cumulative concordance rates successively including different signs of coronary heart disease were compared it was found that the highest monozygotic/dizygotic ratio was 1.73. This ratio was reached when all signs of clinically overt coronary heart disease were included in the calculation (figure 5.2). In a 7 year follow-up by telephone of the 37 (19 monozygotic and 18 dizygotic) pairs that had been discordant with respect to the presence of clinically overt coronary heart disease it was found that 9 monozygotic individuals and 7 dizygotic individuals had developed symptoms of coronary heart disease during the intervening period thus increasing concordance even further (Liljefors 1974).

5.3.2 Biological risk factors

As was pointed out in the literature review the influence of heredity on coronary heart disease is probably of a polygenic nature and consequently many of the biological risk factors would dislodge a genetic dependence. Of all the clinical twin studies the one by Lundman (1966) on 196 twin pairs is deemed the most suitable for evaluation of the genetic influence of biological risk factors such as blood pressure and serum lipids on the development of coronary heart disease.

Analyses of variance were performed for both systolic and diastolic blood pressure and significant F ratios were found for the monozygotic/dizygotic intrapair variance for both males and females. This shows that the variability in blood pressure is greatly influenced by genetic factors.

When analyses of the same type were applied to serum lipid it was found that the variability of phospholipids was under a strong genetic influence. For cholesterol the intrapair variances for monozygotic and

SMOKING AND ITS ENVIRONMENTAL AND HEREDITARY INTERACTIONS

§ 1 Introduction

It has long and repeatedly been suggested that smokers differ from non-smokers in regard to some structural as well as behavioral characteristics other than smoking. Statistics have revealed that smoking habits are different in different population strata defined for example by sex, age and thin background (Friedman et al 1972). Smoking has traditionally been regarded as a mal trait. In Sweden smoking was regarded as socially incorrect behavior for women until about 20-30 years ago. Over time this norm has changed. The younger generations of females have clearly taken up the smoking habit more and more. This seems to be part of a cultural pattern and smoking behavior as such has not been suggested to be biologically linked to sex or to age.

It is also apparent from the literature that smoking is more common in urbanized areas than in rural ones. Furthermore, certain educational and occupational categories show relatively higher number of smokers than do others (Swedish Central Bureau of Statistics 1965; Maccannel, Khushf and Mills 1956; Cederlöf et al 1975). This may be linked to financial considerations but more likely to life-style factors linked to social inheritance. The higher prevalence of smoking in some groups may to some extent have a genetic background as it can be reasonably argued that people of certain personality type would prefer to move from the country to the city from the lower educational levels of their parents to higher educational levels and that such a personality type would have certain ambitions as to occupation.

During the 1950's that is during the time when the first large British and American prospective smoking study began to appear in the literature - some reports were published that suggested differences between smokers and nonsmokers not only in regard to population characteristics and socioeconomic data but also in regard to physiological, behavioral and personality variables (Weath 1958; Schubert 1958; Lillienfeld 1959). Fisher (1958, 1959) advanced his controversial theory that differences between smokers and nonsmokers could be of genetic origin and could be associated with other genetic mechanisms.

More recent reports have documented associations between smoking and several characteristics that are likely to compete with smoking as risk factors for illness or premature death. Higgins and Kjelsberg (1967) studied smokers and nonsmokers in regard to social characteristics and physiological variables. They found that smokers consume more alcohol, have ages than nonsmokers. This finding was later confirmed by ourselves using the American twin registry (Wrubec et al 1973), the old Swedish twin registry (Friberg et al 1973) and smoking study on probability sample of 55 000 Swedish men and women (Cederlöf et al 1975).

Associated with the Kaiser Permanente Research Institute in USA (cf Friedman et al 1972, 1974) an American research team has published a series of articles during the last few years comparing smokers and

DEATH CONCORDANCE RATES
MALES born 1901 1925

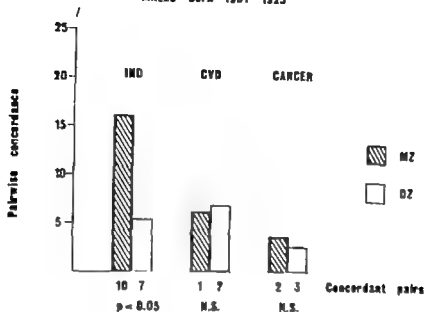


Figure 5.3 Death concordance rates for twin pairs born during 1901-1925 males (From de Faire et al. 1975)

Since 1971 the Swedish Twin Registry has been matched monthly against the mortality records for all Sweden. This has allowed a medical examination of the co-twins of deceased partners within a reasonable time limit (de Faire 1974). Up to 1973 262 pairs became death discordant and 205 of the survivors underwent medical examination where especially clinical signs of coronary heart disease were searched for. If there is a genetic component in coronary heart disease the prevalence of coronary heart disease should be high in the most genetically predisposed co-twins, that is in the monozygotic co-twins whose partners had died from coronary heart disease. Those co-twins whose partners had died from other causes should have a lower prevalence of coronary heart disease. The prevalence of myocardial infarction, angina pectoris and pathological Q-wave in the ECG in the examined twins was 4/10 in the male monozygotic group whose partners had died from coronary heart disease against 4/25 in the dizygotic group. The prevalence was non-significantly lower in the group whose partners had died from other causes. If electrocardiographic signs of coronary heart disease were also included nearly all monozygotic co-twins were affected. A statistical difference between monozygotic twins whose partners died from coronary heart disease and monozygotic twins whose partners had died from other causes was also found.

6 SMOKING AND ITS ENVIRONMENTAL AND HEREDITARY INTERACTIONS

6.1 Introduction

It has long and repeatedly been suggested that smokers differ from non-smokers in regard to some structural as well as behavioral characteristics other than smoking. Statistics have revealed that smoking habits are different in different population strata defined for example by sex, age and ethnic background (Friedman et al 1972). Smoking has traditionally been regarded as a male trait. In Sweden smoking was regarded as socially incorrect behavior for women until about 20-30 years ago. Over time this norm has changed. The younger generations of females have clearly taken up the smoking habit more and more. This seems to be part of cultural pattern and smoking behavior as such has not been suggested to be biologically linked to sex or to age.

It is also apparent from the literature that smoking is more common in urbanized areas than in rural ones. Furthermore, certain educational and occupational categories show relatively higher number of smokers than do others (Swedish Central Bureau of Statistics 1965; Samer 1966; Shickin and Waller 1956; Cederlöf et al 1975). This may be linked to financial considerations but more likely to life-style factor linked to social inheritance. The higher prevalence of smoking in some groups may to some extent have genetic background as it can be reasonably argued that people of certain personality type would prefer to move from the country to the city from the lower educational levels of their parents to higher educational level and that such personality type would have certain ambitions as to occupation.

During the 1950s that is during the time when the first large British and American prospective smoking studies began to appear in the literature some reports were published that suggested differences between smokers and non-smokers not only in regard to population characteristics and socioeconomic data but also in regard to physiological, behavioral and personality variables (Heath 1958; Schubert 1958; Lilienfeld 1959). Fisher (1958-1959) advanced his controversial theory that differences between smokers and non-smokers could be of genetic origin and could be associated with other genetic mechanisms.

More recent reports have documented associations between smoking and several characteristics that are likely to compete with smoking as risk factors for illness: premature death (Higgins and Kjelsberg 1967), studied smoker and non-smoker in regard to social characteristics and physiological variables. They found that smokers consume more alcoholic beverages than non-smokers. This finding was later confirmed by ourselves using the American twin registry (Hrubec et al 1973), the old Swedish twin registry (Friberg et al 1973) and smoking study on probability sample of 51 000 Swedish men and women (Cederlöf et al 1975).

Associated with the Kaiser Permanente Research Institute in USA (of e.g. Friedman et al 1972-1974) an American research team has published several articles during the last few years comparing smoker and

nonsmokers in regard to exposure to occupational risk factors (Friedman Siegelau and Seltzer 1973) consumption of alcoholic beverages and coffee (Friedman Siegelau and Seltzer 1974) use of oral contraceptives and drugs (Seltzer Friedman and Siegelau 1974) etc The results were based on large samples and in many respects showed obvious differences between smokers and nonsmokers

Possible psychosomatic differences between smokers and nonsmokers have been observed in regard to for example pain tolerance (Seltzer et al 1974) and electroencephalographic pattern (Brown 1973) Probably the most well-known psycho-social characteristics related to smoking are the so-called type A and type B behavioral patterns which were first described by Friedman and Rosenman (1959) On the basis of his personality inventory Eysenck (1965) reported that smokers have a more pronounced extraverted behavior than nonsmokers Floderus (1974) modified Eysenck's personality inventory for use in connection with mailed questionnaire investigations She showed among other things that the neuroticism dimension of the scale is also clearly related to smoking in such a way that smokers show on an average a more unstable psychological status

6 2 Structural and Behavioral Characteristics of the Smoker

6 2 1 Introductory remarks

Apart from first section presenting smoking in relation to sex and age the present part of the chapter will report data on the relation between smoking and a series of behavioral characteristics. Data have been taken mainly from the new Swedish twin registry covering the cohorts born 1926 to 1938 and occasionally from the older part of the registry covering the cohorts born 1901 to 1925.

Data will be presented in both A-series and B-series fashion. The A-series analysis will disclose the generally appearing difference between smokers and nonsmokers as unrelated individuals. The B-series analysis aims at disclosing to what extent A-series ratios between smokers and nonsmokers prevail or become altered in the B-series indicating the degree of control or the gain in group comparability achieved by using twin pairs (cf section 2 2 2). For the last mentioned analysis only part of the available pairs have been used, namely those pairs where both partners are concordant nonsmoker or concordant present cigarette smokers or where the one member is a nonsmoker and the other is present regular smoker. This restriction has been made because smoking amount is difficult to assess in groups where the cigarette smoking is combined with other types of smoking. Furthermore, there is no unobjectionable way to equalize numbers of cigarettes to different amounts of pipe smoking. Former smokers have also been excluded due to the problems they would pose in regard to quantitative assessment.

In the following sections the A-series tables will present prevalence rates among nonsmokers by sex and age as well as ratios of the prevalence rates in different smoking groups over the prevalence rate of nonsmokers. All rates are age-adjusted on the basis of five-year intervals, the weights being based on the age distribution among nonsmokers. The statistical testing is performed on the 5-percent level and on the directed alternate hypothesis that ratios are above unity. The A-series tables are denoted by an A after the table number.

The B-series tables display only ratios which are adjusted for differences with regard to sex, age and smoking distribution in the subgroupings, the weights being based on the distributions of sex, age and smoking in the combined discordance groups. The statistical testing is also here performed on the 5-percent level and on the directed alternate hypothesis that the discordance groups show lower ratios than the ratio of concordant smokers over concordant nonsmokers. The B-series tables have the same number as the corresponding A-series tables but are denoted by a B.

The numbers 1 and 2 in the A- and B-series analyses are presented in table 1A and 6B, page 64 respectively.

Table 6 1A Numbers at Risk by Sex and Age in Different Smoking Groups; New Swedish Twin Registry A-series

		Non- Born smokers	Cigarettes only			Ciga- rettes and pipe	Pipe Former and pipe only smokers	
			<7	8-15	>16			
Males	1956-58	1037	67	65	7	78	18	103
	1946-55	1758	250	588	246	853	179	543
	1926-45	2831	339	743	577	1130	676	1346
Females	1956-58	1036	208	150	12	-	-	103
	1946-55	2173	682	1251	296	-	-	652
	1926-45	4875	815	1761	540	-	-	1207

Table 6 1B Numbers at Risk in Different Smoking Concordance and Discordance Groups; New Swedish Twin Registry B-series

Group	No of Pairs
Concordant Nonsmokers	4117
Concordant Smokers (Present cigarette smokers only)	1770
Discordant Smokers (Present cigarette smokers vs nonsmokers)	1350
- Monozygotes	340
- Dizygotes	1010

6 2 2 Selected data on education and employment

It appears from table 6 2A that among present smokers a significant number has a lower education than nonsmokers while there is no relation between former smoking and educational level. The ratios do not show any convincing relations to amount smoked nor do they indicate differences between age and sex groups. The B-series groups (table 6 2B page 66) deviate significantly from the A-series ratio 1.24 and are close to unity regardless of zygosity.

All items concerning employment show significant relations to present smoking and in several subgroups also to former smoking. The highest and markedly dose related ratios are found for employment in the age group born 1936-58. By and large shiftwork and piecework seem to be dose related in most but not all subgroups over time. The values in the B-series analysis (table 6 2B) do not show any control.

Tabl 6 2A Selected Data on Education and Employment by Sex and Age; Age-adjusted Ratios in Smoking Groups in Relation to Prevalence Rates Among Non-smokers; New Swedish Twin Registry A-series

	Born	Non-smokers Prevalence Rate	Cigarettes only			Ciga- rettes and pipe	Pipe Former	
			<7	8-15	>16		only	smokers
<hr/>								
Elementary school only								
Males	1946-55	29.3	1.2	1.4	1.3	1.2	1.0	1.0
	1926-45	44.3	1.2	1.3	1.1	1.2	1.2	0.9
Females	1946-55	25.0	1.3	1.6	1.6			0.9
	1926-45	51.2	1.1	1.1	1.1			0.9
Employed								
Males	1956-58	7.5	2.2	3.9	(9.5)	4.4	(3.7)	1.4
	1946-55	58.2	1.2	1.3	1.2	1.2	1.0	1.2
	1926-45	88.4	1.0	1.0	1.0	1.0	1.0	1.0
Females	1956-58	3.3	4.4	6.5	(17.7)		-	2.6
	1946-55	55.2	1.1	1.1	1.3			1.0
	1926-45	60.5	1.1	1.1	1.2			1.0
Overtime								
Males	1946-55	19.4	0.9	1.1	1.7	1.5	1.3	1.1
	1926-45	32.8	1.0	0.9	1.3	1.1	0.9	1.1
Females	1946-55	10.2	1.0	1.0	1.4			1.2
	1926-45	8.9	1.0	1.1	2.0			1.1
Shiftwork								
Males	1946-55	19.2	1.2	1.4	1.5	1.6	1.6	1.1
	1926-45	19.2	1.2	1.2	1.4	1.6	1.7	1.3
Females	1946-55	9.7	1.4	1.4	2.0			1.3
	1926-45	9.0	1.2	1.6*	1.6			1.2
Piecework								
Males	1946-55	19.6	1.1	1.2	1.3	1.3	1.1	1.2
	1926-45	38.2	1.3	1.2	1.0	1.3	1.2	1.1
Females	1946-55	11.8	1.2	1.3	1.8			1.4
	1926-45	12.6	1.2	1.6	1.7			1.1

Table 6 2B Selected Data on Education and Employment; Sex and Age Standardized Ratios between Smokers and Nonsmokers in Concordant Groups in Comparison to Discordant Groups by Zygosity; New Swedish Twin Registry B-series

	Concordant Smokers versus Concordant Nonsmokers	Smokers versus Nonsmokers in Discordant Groups	
		DZ	MZ
Elementary school only	1.24	1.03	1.96
Employed	1.06	1.09	1.10
Overtime	0.86	1.41	1.23
Shiftwork	1.27	1.11	1.04
Piecework	1.17	1.13	1.27

6 2 3 Selected data on alcohol consumption

In the A-series analysis both drinking and excessive drinking (table 6 3A) turn out to be strongly correlated to smoking in all smoking groups. The dose gradient is most clearly seen for excessive drinking but also exists especially in the youngest age groups for alcohol drinking in total. There is an obvious relation to age for both items which also shows up among cigarette plus pipe smokers and pipe only smokers. In most subgroups former smokers show rates similar to the lowest amount group among present smokers but the relation is significant throughout.

The control of the alcohol items is strong and significant for both dizygotic and monozygotic twins (table 6 3B). Although the control is significant meaning that the ratios in the zygosity groups are reliably lower than those found between concordant smokers and concordant non smokers, the ratios especially for excessive drinking are obviously well above unity. The B-series analysis from the cohorts 1901-1925 is also included in the table and the same tendency can be seen for them.

Table 6 3A Selected Data on Alcohol Consumption by Sex and Age; Age-adjusted Ratios in Smoking Groups in Relation to Prevalence Rates Among Nonsmokers; New Swedish Twin Registry A-series

	Born	Non-smokers Prevalence Rate	Cigarettes only			Ciga- rettes and pipe	Pipe Former only smokers	
			<7	8-13	>16			
			Ratio				Ratio	
<hr/>								
Alcohol drinking								
Males	1956-58	13.1	3.2	5.9	(5.4)	5.2	(5.9)	3.8
	1946-55	66.5	1.3	1.4	1.4	1.4	1.4	1.3
	1926-45	67.1	1.3	1.3	1.4	1.4	1.3	1.3
Female	1956-58	17.8	3.3	4.2	(3.7)			2.9
	1946-55	57.8	1.4	1.4	1.5			1.3
	1926-45	46.6	1.4	1.5	1.7			1.5
Excessive alcohol drinking								
Males	1956-58	2.0	9.0	16.9	(14.3)	22.5	(19.5)	7.3
	1946-55	13.3	2.6	2.9	3.2	3.3	2.4	2.5
	1926-45	5.5	3.2	3.5	5.1	4.0	2.6	2.2
Female	1956-58	1.1	9.2	21.2	(30.3)			7.1
	1946-55	1.8	3.7	5.6	12.6			3.5
	1926-45	0.5	4.2	8.6	21.9			5.3

Table 6 3B Selected Data on Alcohol Consumption; Sex and Age Standardized Ratios between Smokers and Nonsmokers in Concordant Groups in Comparison to Discordant Groups by Zygosity; Swedish Twin Registry B-series

	Concordant Smokers versus Concordant Nonsmokers	Smoker versus Nonsmokers in Discordant Groups	
		DE	ME
Born 1926-58			
Alcohol drinking	1.48	1.22	1.09
Excessive alcohol drinking	4.49	1.74	1.49
Born 1901-25			
Alcohol drinking	2.05	1.26	1.22
Excessive alcohol drinking	6.53	2.08	1.72

Table 6 2B Selected Data on Education and Employment; Sex and Age Standardized Ratios between Smokers and Nonsmokers in Concordant Groups in Comparison to Discordant Groups by Zygosity; New Swedish Twin Registry B-series

	Concordant Smokers versus Concordant Nonsmokers	Smokers versus Nonsmokers in Discordant Groups	
		DZ	MZ
Elementary school only	1 24	1 03	0 96
Employed	1 06	1 09	1 10
Overtime	0 86	1 41	1 23
Shiftwork	1 27	1 11	1 04
Piecework	1 17	1 13	1 27

6 2 3 Selected data on alcohol consumption

In the A series analysis both drinking and excessive drinking (table 6 3A) turn out to be strongly correlated to smoking in all smoking groups. The dose gradient is most clearly seen for excessive drinking but also exists especially in the youngest age groups for alcohol drinking in total. There is an obvious relation to age for both items which also shows up among cigarette plus pipe smokers and pipe only smokers. In most subgroups former smokers show rates similar to the lowest amount group among present smokers but the relation is significant throughout.

The control of the alcohol items is strong and significant for both dizygotic and monozygotic twins (table 6 3B). Although the control is significant meaning that the ratios in the zygosity groups are reliably lower than those found between concordant smokers and concordant nonsmokers the ratios especially for excessive drinking are obviously well above unity. The B series analysis from the cohorts 1901-1925 is also included in the table and the same tendency can be seen for them.

6 2 4 Selected data on drug consumption

Among the drugs selected for analysis tonics and vitamins were supposed to be "healthy" and to relate negatively to smoking. This is confirmed to some extent by the outcome among present smokers (table 6 4A). With few exceptions the λ test in almost all subgroups are unity or below unity. The values for the former smokers are never below unity.

Prescription-free analgesics, sleeping pill and tranquillizers are all related to present as well as former smoking. Among present cigarette smokers the relation to amount is most clearly seen for tranquillizers but also exists for the other drugs in this group. There is no obvious sex difference. Although the rates for former smokers on an average are lower than those for smokers with the highest amount of cigarettes they nonetheless are very often increased significantly.

Oral contraceptives are significantly and markedly related to presently well as formerly smoking women. The highest ratios are found in the youngest female group but the prevalence among nonsmokers is rather low throughout. An increased ratio is seen also in women who smoke 7 cigarettes or less a day.

The B-series analysis (table 6 4B) shows a certain amount of control for prescription-free analgesics, sleeping pills and oral contraceptives. The last mentioned drug shows significant, indeed almost ideal, control in both zygosity groups.

Tabl 6 4B Selected Data on Drug Consumption; Sex and Age Standardized Ratios between Smokers and Nonsmokers in Concordant Groups in Comparison to Discordant Groups by Zygosity; New Swedish Twin Registry B-series

	Concordant Smokers versus Concordant Nonsmokers	Smokers versus Nonsmokers in Discordant Groups	
		DZ	MZ
Tonics and vitamins	1.01	0.92	0.94
Prescription-free analgesics	1.28	1.04	1.17
Sleeping pills	2.34	1.36	1.85
Tranquillizers	1.87	1.60	1.77
Oral contraceptives	2.21	1.19	0.98

Table E 4A Selected Data on Drug Consumption by Sex and Age; Age-adjusted Ratios in Smoking Groups in Relation to Prevalence Rates Among Nonsmokers; New Swedish Twin Registry A series

	Born	Non-smokers	Cigarettes only			Ciga- rettes and pipe	Pipe Former only smokers
		Prevalence Rate	<7	8-15	>16		
			Ratio			Ratio	
<hr/>							
Tonics and vitamins							
Males	1956-58	36.8	0.8	0.8	(1.6)	0.7	(1.5) 1.0
	1946-55	26.1	0.8	0.8	0.7	0.9	0.8 1.1
	1926-45	23.4	0.9	0.8	0.6	0.9	0.8 1.1
Females	1956-58	42.3	1.1	1.1	(1.4)	-	- 1.3
	1946-55	48.9	1.0	1.0	0.9	-	- 1.1
	1926-45	49.5	0.9	0.9	0.9	-	- 1.2
Prescription-free analgesics							
Males	1956-58	11.9	1.0	2.6	(2.4)	2.1	(0.9) 1.6
	1946-55	13.2	1.2	1.5	1.7 ^a	1.8	1.4 1.5
	1926-45	21.8	1.1	1.2	1.3	1.3	1.1 1.2
Females	1956-58	25.9	1.3	2.0	(1.0)	-	- 1.5
	1946-55	36.7	1.0	1.1	1.3		- 1.1
	1926-45	37.7	1.0	1.2	1.3		- 1.2
Sleeping pills							
Males	1956-58	0.7	4.3	0.0	(0.0)	3.7	(0.0) 0.0
	1946-55	1.0	3.3	1.1	2.7	3.5	1.7 2.1
	1926-45	2.8	0.8	1.5	2.2	2.3	1.4 1.2
Females	1956-58	0.9	1.6	6.7	(0.0)	-	- 2.1
	1946-55	2.2	1.3	2.0	5.0		- 1.8
	1926-45	5.2	1.7	2.1	3.4		- 1.5
Tranquillizers							
Males	1956-58	1.7	0.9	1.8	(8.4)	3.8	(0.0) 0.0
	1946-55	3.5	1.5	1.1	2.1	1.8	1.4 1.8
	1926-45	5.8	1.6	1.8	2.1	2.1	1.5 1.5
Females	1956-58	2.7	1.4	2.7	(3.1)	-	- 1.8
	1946-55	5.6	1.4	1.9	3.6	-	- 1.6
	1926-45	13.9	1.3	1.6	2.4	-	- 1.3
Oral contra- ceptives							
Females	1956-58	1.8	4.0	8.9	(9.3)		- 4.8
	1946-55	19.8	1.5	1.7	1.7		- 1.3
	1926-45	11.3	1.3	1.6	2.0		- 1.4

6 2 4 Selected data on drug consumption

Among the drugs selected for analysis tonics and vitamins were supposed to be "healthy" and to relate negatively to smoking. This is confirmed to some extent by the outcome among present smokers (table 6 4A). With few exceptions the rates in almost all subgroups are unity or below unity. The values for the former smokers are never below unity.

Prescription-free analgesics, sleeping pills and tranquillizers are all related to present as well as former smoking. Among present cigarette smokers the relation to amount is most clearly seen for tranquillizers but also exists for the other drugs in this group. There is no obvious sex difference. Although the rates for former smokers on an age age are lower than those for smoker with the highest amount of cigarettes they nonetheless are very often increased significantly.

Oral contraceptives are significantly and markedly related to presently as well as formerly smoking women. The highest rates are found in the youngest female group but the prevalence among nonsmokers is rather low throughout. An increased rate is seen also in women who smoke 7 cigarettes or less a day.

The B-series analysis (table 6 4B) shows a certain amount of control for prescription-free analgesics, sleeping pills and oral contraceptives. The last mentioned drug shows significant indeed almost ideal control in both zygosity groups.

Table 6 4B Selected Data on Drug Consumption; Sex and Age Standardized Ratios between Smokers and Nonsmokers in Concordant Groups in Comparison to Discordant Groups by Zygosity; New Swedish Twin Registry B-series

	Concordant Smokers versus Concordant Nonsmokers	Smokers versus Nonsmokers in Discordant Groups	
		DD	MM
Tonics and vitamins	1.01	0.92	0.94
Prescription-free analgesics	1.20	1.04	1.17
Sleeping pills	2.34	1.36	1.83
Tranquillizers	1.87	1.60	1.77
Oral contraceptives	2.21	1.19	0.98

Table 6 4A Selected Data on Drug Consumption by Sex and Age; Age-adjusted Ratios in Smoking Groups in Relation to Prevalence Rates Among Nonsmokers; New Swedish Twin Registry A-series

	Born	Non-smokers	Cigarettes only			Cigarettes and pipe	Pipe only	Former smokers
			<7	8-15	>16			
		Prevalence Rate	Ratio			Ratio		
<hr/>								
Tonics and vitamins								
Males	1956-58	36 0	0 8	0 8	(1 6)	0 7	(1 5)	1 0
	1946-55	26 1	0 8	0 8	0 7	0 9	0 8	1 1
	1926-45	23 4	0 9	0 8	0 6	0 9	0 8	1 1
Females	1956-58	42 3	1 1	1 1	(1 4)	-	-	1 3
	1946-55	48 9	1 0	1 0	0 9	-	-	1 1
	1926-45	49 5	0 9	0 9	0 9	-	-	1 2
Prescription free analgesics								
Males	1956-58	11 9	1 0	2 6	(2 4)	2 1	(0 9)	1 6
	1946-55	13 2	1 2	1 5	1 7	1 8	1 4	1 5
	1926-45	21 8	1 1	1 2	1 3	1 3	1 1	1 2
Females	1956-58	25 9	1 3	2 0	(1 0)	-	-	1 5
	1946-55	36 7	1 0	1 1	1 3	-	-	1 1
	1926-45	37 7	1 0	1 2	1 3	-	-	1 2
Sleeping pills								
Males	1956-58	0 7	4 3	0 0	(0 0)	3 7	(0 0)	0 0
	1946-55	1 0	3 3	1 1	2 7	3 5	1 7	2 1
	1926-45	2 8	0 8	1 5	2 2	2 3	1 4	1 2
Females	1956-58	0 9	1 6	6 7	(0 0)	-	-	2 1
	1946-55	2 2	1 3	2 0	5 0	-	-	1 8
	1926-45	5 2	1 7	2 1	3 4	-	-	1 5
Tranquillizers								
Males	1956-58	1 7	0 8	1 8	(8 4)	3 8	(0 0)	0 0
	1946-55	3 5	1 5	1 1	2 1	1 8	1 4	1 8
	1926-45	5 8	1 6	1 8	2 1	2 1	1 5	1 5
Females	1956-58	2 7	1 4	2 7	(3 1)	-	-	1 8
	1946-55	5 6	1 4	1 9	3 6	-	-	1 6
	1926-45	13 9	1 3	1 6	2 4	-	-	1 3
Oral contraceptives								
Females	1956-58	1 8	4 8	8 9	(9 3)	-	-	4 8
	1946-55	19 6	1 5	1 7	1 7	-	-	1 3
	1926-45	11 3	1 3	1 6	2 0	-	-	1 4

6 2 4 Selected data on drug consumption

Among the drugs selected for analysis tonics and vitamins were supposed to be "healthy" and to relate negatively to smoking. This is confirmed to some extent by the outcome among present smokers (table 6 4A). With few exceptions the rates in almost all subgroups are unity or below unity. The values for the former smokers are here below unity.

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Tabl 6 4B Selected Data on Drug Consumption; Sex and Age Standardised Ratios between Smokers and Nonsmokers in Concordant Groups in Comparison to Discordant Groups by Zygosity; New Swedish Twin Registry B-series

	Concordant Smokers versus Concordant Nonsmokers	Smokers versus Nonsmokers in Discordant Groups	
		DE	ME
Tonics and vitamins	1.01	0.92	0.94
Prescription-free analgesics	1.28	1.04	1.17
Sleeping pills	2.34	1.36	1.83
Tranquillizers	1.87	1.88	1.77
Oral contraceptives	2.21	1.19	0.98

Table E 5A Selected Data on Psycho-social Items by Sex and Age; Age-adjusted Ratios in Smoking Groups in Relation to Prevalence Rates Among Nonsmokers; New Swedish Twin Registry A-series

	Born	Non smokers Prevalence Rate	Cigarettes only			Ciga- rettes and pipe	Pipe only	Former smokers
			<7	8-15	>16			
			Ratio			Ratio		
<hr/>								
Instability								
Males	1956-58	11.2	1.6	2.1	3.8	2.1	2.0	1.6
	1946-55	13.9	1.5	1.5	2.2	1.7	1.5	1.6
	1926-45	12.0	1.6	1.6	1.8	1.8	1.6	1.3
Females	1956-58	23.0	2.1	2.2	1.8	-	-	1.7
	1946-55	24.8	1.3	1.7	2.2	-	-	1.3
	1926-45	22.3	1.4	1.5	2.0	-	-	1.2
Extravertness								
Males	1956-58	69.7	1.2	1.0	1.2	1.2	1.2	1.0
	1946-55	60.7	1.1	1.1	1.1	1.1	1.0	1.0
	1926-45	55.6	1.2	1.1	1.2	1.1	1.0	1.0
Females	1956-58	54.7	1.1	1.1	1.2	-	-	1.2
	1946-55	46.2	1.2	1.2	1.2	-	-	1.1
	1926-45	42.9	1.2	1.2	1.3	-	-	1.1
Sleeping difficulties								
Males	1956-58	19.9	1.5	2.0	1.4	1.7	1.4	1.4
	1946-55	11.9	1.6	1.8	2.3	1.7	2.2	1.3
	1926-45	10.2	1.4	1.7	1.9	2.1	1.6	1.2
Females	1956-58	26.3	1.4	1.8	1.6	-	-	1.3
	1946-55	17.2	1.4	1.6	2.2	-	-	1.1
	1926-45	15.4	1.4	1.6	2.3	-	-	1.2
Stress								
Males	1956-58	9.2	0.7	0.7	1.6	2.1	0.6	1.5
	1946-55	13.6	1.2	1.2	1.9	1.4	1.4	1.0
	1926-45	17.5	1.1	1.2	1.5	1.3	1.3	1.2
Females	1956-58	11.0	1.3	1.3	1.5	-	-	1.0
	1946-55	13.4	1.0	1.2	1.7	-	-	1.0
	1926-45	11.1	1.2	1.4	2.1	-	-	1.2
Divorced								
Males	1946-55	0.4	0.0	2.0	4.0	1.8	1.1	0.8
	1926-45	2.7	2.3	2.3	4.0	2.8	1.2	0.9
Females	1946-55	0.7	0.8	2.8	4.3	-	-	1.4
	1926-45	3.9	1.1	2.4	4.4	-	-	1.2

Tabl 6 5A. (continued)

	Born	Non smokers	Cigarettes only			Ciga- rettes and pipe	Pipe only	Former smokers
			<7	8-15	>16			
		Prevalence Rate	Ratio			Ratio		
<hr/>								
Change of em- ployer >3 times								
Males	1946-55	20.6	1.5	1.8	2.1	1.8	1.6	1.5
	1926-45	16.5	1.3	1.4	1.3	1.3	1.3	1.3
Females	1946-55	25.2	1.1	1.3	1.7			1.2
	1926-45	11.7	1.1	1.2	1.4			1.3
Low physical activity								
Males	1956-58	6.2	3.4	4.0	(5.9)	4.5	(0.9)	2.2
	1946-55	16.0	1.2	1.9	2.8	1.9	1.6 ⁺	1.2
	1926-45	17.2	1.1	1.5	2.3	1.7	1.3	1.0
Females	1956-58	9.7	1.7	3.3	(4.3)			2.0
	1946-55	20.8	1.2	1.8	2.4		-	1.2
	1926-45	22.8	1.0	1.5	2.1		-	0.9
Cooked food once day or less								
Males	1956-58	5.3	1.4	3.2	(5.4)	3.2	(2.1)	0.9
	1946-55	27.8	1.2	1.3	1.4	1.2	1.2	1.0
	1926-45	37.0	1.1	1.2	1.2	1.1	1.1	1.0
Females	1956-58	11.2	1.5	2.5	(3.0)			1.0
	1946-55	50.9	1.1	1.1	1.3			1.0
	1926-45	41.1	1.2	1.2	1.4			1.1
Coffee > 3 cups day								
Males	1956-58	4.5	2.3	3.1	(0.0)	2.8	(4.9)	1.9
	1946-55	16.2	1.2	2.0	2.8	2.5	2.1 ⁺	1.6
	1926-45	32.2	1.3	1.6	1.9	1.8	1.8	1.3
Females	1956-58	4.1	3.2	4.4	(10.2)			2.1
	1946-55	12.6	1.4	2.7	4.2 ⁺			1.6
	1926-45	32.6	1.2	1.7	2.0			1.2

6 2 5 Selected data on psycho-social and related items

Among the items analyzed (Table 6 5A) only two are psycho-social in the narrow sense of emanating from psycho-metric scaling namely instability and extravertness. Some other items indicate unstable behavior that might be personality related such as jumping difficulties stress and divorce. Frequent change of employer low physical activity cooked food once day or less and 5 cups of coffee a day or more may be assumed to relate in some way to psycho-social factors.

Table 6 5B Selected Psycho-socially" Related Items; Sex and Age Standardised Ratios between Smokers and Nonsmokers in Concordant Groups in Comparison to Discordant Groups by Zygosity; New Swedish Twin Registry B-series

	Concordant Smokers versus Concordant Nonsmokers	Smokers versus Nonsmokers In Discordant Groups	
		DZ	MZ
Instability	1 81	1 32	1 18
Extravertness	1 15	1 20	1 01
Sleeping difficulties	1 77	1 39	1 29
Stress	1 36	1 19	1 02
Divorced	2 70	1 85	1 25
Change of employer			
≥3 times	1 40	1 15	0 81
Low physical activity	1 64	1 57	1 20
Cooked food once a day or less	1 32	1 96	1 17
Coffee ≥5 cups a day	1 75	1 90	1 44

It is obvious that whatever item is studied positives occur more frequently among present smokers and in some cases among former smokers as well. With the exception of extravertness all items are also related to amount of cigarettes smoked. These tendencies are strongest for divorce and instability. There are no obvious sex or age differences except possibly a tendency to higher ratios in the youngest cohort.

It is noteworthy that most of these items are rather well controlled in the B-series (table 6 5B). Instability shows a decrease from 1 8 to 1 2 which is significant. Divorce is also markedly controlled in the B-series.

6 3 Hereditary Aspects

6 3 1 Introductory remarks

The degree of control or in other terms the increased comparability between smokers and nonsmokers in twin pairs is most certainly an effect of early environment the "social heredity" but may also be a result of true genetic determinants. One way to discriminate between an environmental and a genetic influence is to use the so-called classical twin method meaning a comparison of monozygotes and dizygotes in regard to concordance rates. In the case of complete ascertainment (when all twin pairs including the concordantly negatives have been observed) the concordance rate can be replaced by the coincidence rate (cf section 2 1 2). It is also possible to calculate an expected coincidence rate on the assumption of random combinations. This ratio between observed and expected coincidence is the gross effect of social and genetic heredity. In the statistical testing the chi-square test for heterogeneity has been used whenever the ratio is high for monozygotic twins some extra resemblance between the partners in such pairs indicated. This extra resemblance may be due either to genetic influences or to a social heredity that is stronger for monozygotic than for dizygotic twins. The difference between monozygotic and dizygotic pairs is regarded to the strength of the association has been evaluated by a statistical test of third-order interaction (Kastenbaum and Lapham 1959). The difference is expressed as quotient between the ratios for MZ and DZ respectively.

The following sections of the present chapter show coincidence rates for 11 items presented in earlier sections. Furthermore attempts have been made to test the hypothesis that the habitual items should be dependent on social and/or genetic heredity and hence also show increased ratios among smokers in twin pairs where the partner is a smoker compared to ratios among nonsmokers in concordantly nonsmoking pairs (MZ-analysis).

6 3 2 Heredity of smoking

Observations that the smoking habit is more concordant in monozygotic than in dizygotic twins have been reported by Friberg et al (1959) by Shields (1962) and by Cederlöf (1966) in a publication based on the old Swedish twin registry.

Table 6 page 74 shows that with one single exception 11 sex and age groups have significantly increased ratios for observed over expected coincidence rates. It is also seen that the quotients are significantly higher for monozygotic than for dizygotic twins which may be interpreted as evidence of genetic component or of stronger environmental pressure for conformity.

6 3 3 Selected data on education and employment

The amount of formal education as well as different items related to employment show observed coincidence ratios that are significantly greater than expected throughout (table 6 7 page 74). Consistently the quotients are significantly higher among monozygotes as compared to dizygotes.

The hypothesis that education and employment items in the nonsmoking partners should be related to the smoking status of the co-twin (MZ analysis) did not receive support (table 6 8 page 75).

Table 6 5B Selected "Psycho-socially" Related Items; Sex and Age Standardized Ratios between Smokers and Nonsmokers in Concordant Groups in Comparison to Discordant Groups by Zygosity; New Swedish Twin Registry B-series

	Concordant Smokers versus Concordant Nonsmokers	Smokers versus Nonsmokers In Discordant Groups	
		DE	ME
Instability	1 81	1 32	1 18
Extravertness	1 15	1 20	1 01
Sleeping difficulties	1 77	1 39	1 29
Stress	1 36	1 19	1 02
Divorced	2 70	1 85	1 25
Change of employer ≥3 times	1 40	1 15	0 81
Low physical activity	1 64	1 57	1 20
Cooked food once a day or less	1 32	0 96	1 17
Coffee ≥5 cups a day	1 75	1 90	1 44

It is obvious that whatever item is studied positives occur more frequently among present smokers and in some cases among former smokers as well. With the exception of extravertness all items are also related to amount of cigarettes smoked. These tendencies are strongest for divorce and instability. There are no obvious sex or age differences except possibly a tendency to higher ratios in the youngest cohort.

It is noteworthy that most of these items are rather well controlled in the B-series (table 6 5B). Instability shows a decrease from 1 8 to 1 2 which is significant. Divorce is also markedly controlled in the B-series.

Table 6 8 Selected Data on Education and Employment; Sex and Age Standardized Ratios of Observed Over Expected Prevalence Rates Among Non-smokers Grouped by Smoking Status of Co-twin; New Swedish Twin Registry MZT series

			Non-Smokers	Present Cigarette Smokers	All Present Smokers	Former Smokers
No risk	MZ		1881	340	423	270
	DZ		2236	1010	1307	544
Elementary school only	MZ		1 00	1 04	1 04	0 95
	DZ		0 98	1 04	1 03	0 90
Employed	MZ		1 01	0 96	0 97	1 06
	DZ		0 98	1 07	1 07	1 09

6 3 4 Selected data on alcohol consumption

Both items on drinking particularly excessive drinking (table 6 9 page 76) show observed coincidence rates that are far above expectation and considerably higher among the monozygotic twins

The hypothesis that nonsmoking partners of smoking co-twins should have increased ratios is strongly verified for both monozygotic and dizygotic twins (table 6 10 page 78) The ratios for the monozygotes are by far the strongest especially in regard to excessive drinking Increased ratios are also found among partners to former smokers

6 3 5 Selected data on drug consumption

The observed coincidence ratios are markedly higher than those expected for the drug consumption items (table 6 11 page 77) as well most so for tranquilizers The differences between the two zygosity groups are significant throughout

The MZT-analysis (table 6 12 page 77) shows markedly increased ratios for sleeping pills but generally negative results for the other items

6 3 6 Selected data on psycho-social and related items

The coincidence ratios (table 6 13 page 78) are increased throughout and in almost all cases significantly so The quotients are also significant in most cases

In the MZT-analysis (table 6 14 page 79) the nonsmoking partners of presently smoking co-twins show increased prevalence ratios for every item in this category The finding holds true in both zygosity groups

Table E 6 "Heredity" of Smoking; Coincidence Ratios and Quotients between MZ and DZ Ratios by Sex, Age and Zygosity; New Swedish Twin Registry

	Coincidence Ratios Observed/Expected		Quotients
	MZ	DZ	MZ/DZ
Males			
1926-45	1.46	1.20	1.22
1946-55	1.68	1.26	1.33
1956-58	4.00	2.67	1.50
Total	1.61	1.32	1.22
Females			
1926-45	1.71	1.41	1.21
1946-55	1.65	1.33	1.24
1956-58	2.78	2.18	1.28
Total	1.76	1.44	1.22

Table E 7 Selected Data on Education and Employment; Coincidence Ratios and Quotients between MZ and DZ Ratios by Sex and Zygosity; New Swedish Twin Registry

	Coincidence Ratios Observed/Expected		Quotient
	MZ	DZ	MZ/DZ
Elementary school only			
Males	1.73	1.48	1.17
Females	1.83	1.51	1.21
Employed			
Males	1.34	1.33	1.01
Females	1.33	1.26	1.06
Overtime			
Males	2.24	1.86	1.20
Females	4.17	1.89	2.21
Shiftwork			
Males	3.26	2.61	1.25
Females	4.80	2.44	1.97
Piecework			
Males	2.22	1.71	1.30
Females	4.49	3.30	1.36

Table 6 II Selected Data on Education and Employment; Sex and Age Standardized Ratios of Observed Over Expected Prevalence Rates Among Non-smokers Grouped by Smoking Status of Co-twin; New Swedish Twin Registry KET-series

		Non-Smokers	Present Cigarette Smoker	All Present Smokers	Former Smokers
No t risk	ME	1881	340	423	270
	DE	2236	1010	1307	544
Elementary school only	ME	1 00	1 04	1 04	0 93
	DE	0 98	1 04	1 03	0 90
Employed	ME	1 01	0 96	0 97	1 06
	DE	0 98	1 07	1 07	1 09

6 3 4 Selected data on alcohol consumption

Both items on drinking particularly excessive drinking (tabl 6 9 page 78) show observed coincidence ratios that are far above expectation and considerably higher among the monozygotic twins

The hypothesis that non-smoking partners of smoking co-twins should have increased ratios is strongly verified for both monozygotic and dizygotic twins (table 6 10 page 76) The ratios for the monozygotes are by far the strongest especially in regard to excessive drinking Increased ratios are also found among partners to former smokers

6 3 5 Selected data on drug consumption

The observed coincidence ratios are markedly higher than those expected for the drug consumption items (table 6 11 page 77) as well as for tranquilizers The differences between the two zygosity groups are significant throughout

The KET-analysis (tabl 6 12 page 77) shows markedly increased ratios for sleeping pills but generally negative results for the other items

6 3 6 Selected data on psycho-social and related items

The coincidence ratios (tabl 6 13 page 78) are increased throughout and in almost all cases significantly so The quotients are also significant in most cases

In the KET analysis (table 6 14 page 79) the non-smoking partners of presently smoking co-twins show increased prevalence ratios for every item in this category The finding holds true in both zygosity groups

Table # 9 Selected Data on Alcohol Consumption; Coincidence Ratios and Quotients between MZ and DZ Ratios by Sex and Zygosity; New Swedish Twin Registry

	Coincidence Ratios Observed/Expected		Quotients
	MZ	DZ	MZ/DZ
Alcohol drinking			
Males	1 46	1 34	1 09
Females	1 44	1 34	1 07
Excessive alcohol drinking			
Males	4 96	3 30	1 50
Females	13 12	6 12	2 14

Table # 10 Selected Data on Alcohol Consumption; Sex and Age Standardized Ratios of Observed Over Expected Prevalence Rates Among Nonsmokers Grouped by Smoking Status of Co-twin; New Swedish Twin Registry MET-series

		Non-Smokers	Present Cigarette Smokers	All Present Smokers	Former Smokers
No at risk	MZ	1881	340	423	270
	DZ	2236	1010	1307	544
Alcohol drinking	MZ	0 93	1 32	1 36	1 45
	DZ	0 93	1 25	1 23	1 15
Excessive alcohol drinking	MZ	8 80	2 81	3 48	2 28
	DZ	0 66	2 00	2 05	1 19

Tabl 6 11 Selected Data on Drug Consumption; Coincidence Ratios and Quotients between MX and DX Ratios by Sex and Zygosity; New Swedish Twin Registry

	Coincidence Ratios Observed/Expected		Quotients MX/DX
	MX	DX	
Tonics and vitamins			
Males	2 19	1 92	1 14
Females	1 48	1 23	1 20
Prescriptionfree analgesics			
Males	2 57	1 87	1 37
Females	1 63	1 32	1 23
Sleeping pills			
Males	3 84	2 34	1 30
Females	4 64	2 90	1 60
Tranquillizers			
Males	7 64	3 73	2 05
Females	3 29	2 84	1 16
Or 1 contraceptives			
Females	2 94	2 02	1 46

Tabl 6 12 Selected Data on Drug Consumption; Sex and Age Standardized Ratios of Observed Over Expected Prevalence Rate Among Nonsmokers Grouped by Smoking Status of Co-twin; New Swedish Twin Registry MZ-series

		Non-Smokers	Present Cigarette Smoker	All Present Smokers	Former Smokers
No	MX	1881	340	423	270
	DX	2236	1010	1307	344
Tonics and vitamins	MX	1 01	0 97	0 96	1 06
	DX	0 99	1 00	0 99	1 11
Prescriptionfree analgesics	MX	1 00	0 90	1 02	1 16
	DX	0 90	1 02	1 01	0 95
Sleeping pills	MX	0 70	1 42	1 75	1 03
	DX	0 84	1 37	1 33	1 02
Tranquillizers	MX	0 95	1 06	1 16	0 94
	DX	1 02	0 99	1 01	1 02

Table 6 13 Selected Data on Psycho-social and "Psycho-socially Related Items" Coincidence Ratios and Quotients between MZ and DZ Ratios by Sex and Zygosity; New Swedish Twin Registry

	Coincidence Ratios Observed/Expected		Quotients MZ/DZ
	MZ	DZ	
Instability			
Males	3 50	2 04	1 72
Females	2 17	1 49	1 46
Extravertness			
Males	1 22	1 09	1 12
Females	1 44	1 18	1 22
Sleeping difficulties			
Males	2 96	1 34	2 21
Females	2 34	1 42	1 65
Stress			
Males	2 75	2 07	1 33
Females	3 11	1 96	1 59
Divorced			
Males	4 69	4 30	1 09
Females	5 12	2 85	1 80
Change of employment >2 time			
Males	2 49	2 13	1 17
Females	2 67	2 36	1 13
Low physical activity			
Males	2 96	1 71	1 73
Females	2 20	1 54	1 43
Cooked food < once a day			
Males	2 17	1 96	1 11
Females	1 60	1 46	1 10
Coffee > 5 cups a day			
Males	2 59	1 72	1 51
Females	2 30	1 65	1 39

Table 6 14 Selected Data on "Psycho-social and "Psycho-socially" Related Items; Sex and Age Standardized Ratios of Observed Over Expected Prevalence Rates Among Nonsmokers Grouped by Smoking Status of Co-twin; New Swedish Twin Registry MET-series

			Non- Smokers	Present Cigarette Smokers	All Present Smokers	Former Smokers
No E risk	ME		1881	340	423	270
	DE		2236	1010	1307	544
Instability	ME		0 95	1 56	1 46	1 39
	DE		0 98	1 13	1 12	0 85
Extroversion	ME		0 99	1 14	1 17	0 93
	DE		1 01	1 02	1 02	0 97
Sleeping difficulties	ME		0 96	1 39	1 24	1 12
	DE		0 96	1 15	1 12	0 92
Stress	ME		0 91	1 25	1 19	1 23
	DE		0 93	1 11	1 15	1 04
Divorced	ME		0 79	2 83	1 82	0 64
	DE		0 87	1 11	1 18	0 91
Change of employer >3 times	ME		0 94	0 99	1 13	1 21
	DE		0 91	1 22	1 25	1 13
Low physical activity	ME		0 90	1 15	1 23	1 02
	DE		1 02	0 96	1 04	0 79
Cooked food once day or less	ME		1 01	1 06	1 03	1 10
	DE		0 97	1 18	1 14	0 98
>5 cups of coffee day	ME		0 97	1 01	1 18	1 04
	DE		1 01	0 96	0 98	1 08

THE SMOKING RELATED DISEASE PANORAMA

Introduction

The aim of this report has not been to present an extensive survey of the vast literature in the field of tobacco and health. Such surveys have been made continuously by the US Public Health Service starting with the first report to the Surgeon General in 1964. Reviews have also been given by the Royal College of Physicians in London (1962-1971) and most recently by WHO Expert Committee (1975).

The majority of investigations have shown statistical associations between the smoking habit and an extensive series of diseases and causes of death. Even if the earliest epidemiological investigations have been rightfully criticized for being of the retrospective type and being based on nonrandom sampling, their main results have been confirmed in later more sophisticated investigations.

The main target of many earlier investigations was cigarette smoking men, but published investigations during recent years have indicated increased risks for women (Wynder 1972; Silverberg and Rolleb 1973; Born 1974) and for smokers of cigars and pipe (Gsell and Abelin 1972). A recently published investigation from Sweden (Cedarlöf et al 1975) with appropriate techniques of sampling and data collection has demonstrated that within equal smoking groups the hypermortality among women approaches that of men and that pipe and cigar smokers experience a considerably increased risk especially of lung cancer.

Few scientists today seem to question the gathered evidence of a cause and effect relationship between lung cancer and smoking. Also in twin studies there has recently come up evidence that lung cancer is associated with smoking both in monozygotic and dizygotic smoking discordant twin pairs (Cedarlöf and Friberg to be published). These data will be commented upon in section 8.3.3.

As for coronary heart disease a report from the World Health Organization (WHO 1975) considers cigarette smoking an important cause of an increased risk of ischaemic heart disease contributing both to the atherosclerotic process itself and to the condition that precipitates heart attack and determines of fatal outcome. The conclusions are based on epidemiological evidence as well as experimental data mostly in connection with carbon monoxide exposure. A study of particular relevance seems to be the one by Anderson et al 1973 who found that volunteers with coronary heart disease exposed during 4 hours to between 60-120 mg CO/m³ giving rise to carboxyhaemoglobin-levels of 1-5 percent in exercise tests showed symptoms of angina pectoris earlier than when breathing pure air.

In a large scale follow-up on the mortality of British doctors (Doll and Peto 1976) the well-known association between smoking and coronary heart disease was again confirmed. The authors consider the very high mortality rates in young age groups to great extent caused by smoking while they are much more cautious concerning the relationship at higher

ages Here it is considered possible that much of the excess mortality from ischaemic heart disease in this group of men arises not because of an effect of smoking but because smoking correlates with some other factor which contributes directly to the disease. If however smoking is a major cause of ischaemic heart disease in young men it would seem likely that it also contributes to causing the disease in the old.

Multifactorial etiology has been more and more emphasized where the importance of physiological risk factors such as hypertension and hypercholesterol have been pointed out (Epstein 1965, 1967; Keys et al 1972; Björck 1975; Keys 1975). Very little has been investigated in regard to habitual factors such as drinking and drug consumption to which however attention has been drawn more recently (Higgins Kjelsberg and Metzner 1967; Klatzky, Friedman and Siegelman 1973, 1974; Saltzer, Friedman and Siegelman 1974; Friedman et al 1975). On a Swedish population sample (Cederlöf et al 1975) the influence of registration for alcohol abuse as well as urban-rural residence appeared to be related to death from coronary heart disease among nonsmokers as well as smokers.

Although the multifactorial etiology has been well recognized the confounding effects of competing risk factors for the assessment of the causal relationship between smoking and disease have generally been disregarded.

Twin investigations into the questions of tobacco smoking and health not related to the present program are very few in previous literature. The only major ones have been performed on the Danish registry compiled in 1954 (cf section 3.1.3).

A publication by Hauge et al (1968) considered mortality and morbidity in relation to tobacco consumption among 904 smoking discordant same-sexed twin pairs where both partners were alive on January 1, 1959. After 7 years of follow-up 114 deaths were recorded among whom 66 belonged to the heavier smokers and 48 to the lighter. The presentation did not consider zygosity. The excess deaths were mainly caused by coronary occlusion where 27 cases had occurred among heavier smokers as against 13 among lighter smokers. In regard to morbidity there were no noteworthy differences in regard to angina pectoris and non-fatal coronary occlusion while claudication, chronic bronchitis and ulcers were more prevalent among the heavier smokers.

In a short publication in 1970 it was stated by Hauge, Harvald and Reid without any presentation of actual data that neither MZ nor DZ pairs showed any tendency to increased mortality in the heavier smoking co-twins. When the material was subdivided according to cause of death no conspicuous exception from the general pattern was found but the sub-groups were relatively small.

In regard to selected diseases it was stated that fatal coronary occlusions and non-fatal occlusions diagnosed in hospitals showed only a slight and non-significant tendency to be associated with higher tobacco consumption and in MZ pairs where only one co-twin had an occlusion he was equally often the heavier smoking as the light smoking one. Symptoms of angina pectoris and chronic bronchitis however showed significant differences between the smoking groups but it was not reported whether in the monozygotic, the dizygotic or both series.

7 2 Twin Studies Morbidity

7 2 1 Introductory remarks

When the twin program was initiated in 1961 the main objective was the follow-up of mortality. The only feasible way of approaching the individuals in the study was by mailed questionnaires. Little was known at that time as to the possibilities of obtaining valid information on morbidity with the aid of self-administered questionnaires. The British Medical Research Council Committee on the Etiology of Chronic Bronchitis had developed a standardized questionnaire on respiratory symptoms and Rose in England had tried to diagnose ischemic heart pain by the aid of questionnaires in field surveys (of section 4 § 1). Both questionnaires had been designed for use by interviewer and had to be modified to be of any value in mailed questionnaire survey where the number of questions is critical for the response rate.

In spite of the questionable validity it was decided to modify these questions and to use them tentatively together with series of other medical questions in the mailed surveys of 1963 in Sweden and 1967 in both Sweden and the United States. Further questions on morbidity were included in the questionnaire of the new Swedish twin registry (Medlund et al 1977). Details on the phrasing of the questions as well as on some validity aspects are given in Chapter 4.

As complement to the more extensive questionnaire studies a number of clinical investigations on morbidity was performed by Lundman (1966) and Lilj for (1970). The following sections will summarise the results in regard to respiratory symptoms, cardiovascular symptoms and some other medical findings.

7 2 2 Respiratory findings

The operationally defined symptoms cough and prolonged cough (cf section 4 § 1) were investigated in all twin studies. A summarized survey of the findings is given in the A-series fashion in tabl 7 1 page 84. It should be pointed out that the smoking categories differ somewhat between the US and Swedish studies and also between the two Swedish studies.

Independent of sex, age and country both symptoms are clearly related to the number of cigarettes smoked among present smokers. The gradient is consistently steeper for the symptom prolonged cough in comparison to cough although the prevalence is lower. The values for former smokers are lower than those for present smokers throughout but in many subgroups somewhat above unity. If the results from the US and Swedish studies are compared given cigarette consumption is generally associated with higher rates in the Swedish than in the American study. For example males born 1911-1925 and smoking more than 10 cigarettes a day in Sweden display ratio of 6.4 in regard to cough which is the same as for smokers of more than 30 cigarettes in USA. The ratios for "prolonged cough" are in the quoted smoking categories 13.4 in Sweden and 8.1 in USA.

Smoking of cigarettes and pipe and of pipe only also show ratios well above unity which is especially notable for the younger Swedish age group.

ages Here it is considered possible that much of the excess mortality from ischaemic heart disease in this group of men arises not because of an effect of smoking but because smoking correlates with some other factor which contributes directly to the disease If however smoking is a major cause of ischaemic heart disease in young men it would seem likely that it also contributes to causing the disease in the old

Multifactorial etiology has been more and more emphasised where the importance of physiological risk factors such as hypertension and hypercholesterol have been pointed out (Epstein 1965 1967; Keys et al 1972; Björck 1975; Keys 1975) Very little has been investigated in regard to habitual factors such as drinking and drug consumption to which however attention has been drawn more recently (Higgins Kjelsberg and Metzner 1967; Klatsky Friedman and Siegelau 1973 1974; Seltzer Friedman and Siegelau 1974; Friedman et al 1975) On a Swedish population sample (Cederlöf et al 1975) the influence of registration for alcohol abuse as well as urban-rural residence appeared to be related to death from coronary heart disease among nonsmokers as well as smokers

Although the multifactorial etiology has been well recognized the confounding effects of competing risk factors for the assessment of the causal relationship between smoking and disease have generally been disregarded

Twin investigations into the questions of tobacco smoking and health not related to the present program are very few in previous literature The only major ones have been performed on the Danish registry compiled in 1954 (cf section 3 1 3)

A publication by Hauge et al (1968) considered mortality and morbidity in relation to tobacco consumption among 904 smoking discordant same-sexed twin pairs where both partners were alive on January 1 1959 After 7 years of follow-up 114 deaths were recorded among whom 66 belonged to the heavier smokers and 48 to the lighter The presentation did not consider zygosity The excess deaths were mainly caused by coronary occlusion where 27 cases had occurred among heavier smokers as against 13 among lighter smokers In regard to morbidity there were no noteworthy differences in regard to angina pectoris and non-fatal coronary occlusion while claudication chronic bronchitis and ulcers were more prevalent among the heavier smokers

In a short publication in 1970 it was stated by Hauge Harvald and Reid without any presentation of actual data that neither MZ nor DX pairs showed any tendency to increased mortality in the heavier smoking co-twins When the material was subdivided according to cause of death no conspicuous exception from the general pattern was found but the subgroups were relatively small

In regard to selected diseases it was stated that 11 fatal coronary occlusions and non-fatal occlusions diagnosed in hospitals showed only a slight and non-significant tendency to be associated with higher tobacco consumption and in MZ pairs where only one co-twin had an occlusion 11 was equally often the heavier smoking as the light smoking one Symptoms of angina pectoris and chronic bronchitis however showed significant differences between the smoking groups but it was not reported whether in the monozygotic the dizygotic or both series

Table 7.2 "Cough and "Prolonged Cough by Sex Age and Smoking Discordance Groups in the Old Swedish and the American Twin Registries B-series

		DIZYGOTES			MONOZYGOTES		
Born		No of Pair	Pooled Low Group	Pooled High Group	No of Pairs	Pooled Low Group	Pooled High Group
SWEDISH REGISTRY							
"Cough							
Males	1911 25	408	16	60	155	12	17
	1901 18	170	13	29	62	1	13
Females	1911 25	514	26	63	235	19	32
	1901 10	165	15	32	53	5	10
"Prolonged cough							
Males	1911 25	408	2	20	155	5	11
	1901 10	170	6	11	62	0	5
Females	1911 25	514	10	27	235	5	10
	1901 10	165	4	12	53	1	3
AMERICAN REGISTRY							
Cough							
Males	1917 27	725	52	122	493	30	74
Prolonged cough							
Males	1917 27	725	32	80	493	21	47

The B-series analyses covering the total pooled low and high groups only is shown in table 7.2 There is consistent difference between the two exposure groups both among dizygotes and monozygotes. The ratios (not displayed in the table) are about the same in both zygosity groups. Although the rates are clearly dependent on age, there is no consistent interaction between age and smoking effect. Nor is any difference seen between males and females. Despite higher smoking discordance in the American registry on an average about 17 opposed to 7 cigarettes in the Swedish study the ties are if any thing less in the American series than in the Swedish.

The symptom cough in the old Swedish twin registry was also evaluated in interaction with smoking and urban-rural residential history (Ceder 1966). The prevalence of respiratory symptoms was higher among concordantly smoking men in urban than in rural areas in the A-analysis as well as in the B-analysis. In concordant monozygotes however there was no difference at all between the areas. This was interpreted as speaking in favor of specific urban factor interacting with smoking.

This problem was further elucidated in the study on American twins (Struhs et al 1973). A series of indices to be correlated with respiratory symptoms was developed on the basis of questionnaire reports on residential history and official US statistics with regard to air pollution.

Table 7 1 Age-adjusted Morbidity Ratios for Cough and Prolonged Cough by Sex Age and Smoking Groups in the Swedish and American Twin Registries (Prevalence among Nonsmokers) A-series

		No at Risk		Cough		Prolonged Cough	
		Males	Females	Males	Females	Males	Females
SWEDISH REGISTRIES							
Born 1946-55							
Nonsmokers		1758	2173	3 0	2 8	- ¹	-
Present cpts	< 7	250	682	2 1*	2 2*	-	-
	8-15	588	1251	2 7	3 7	-	-
	>16	246	296	4 4	8 0	-	-
Cigarettes and pipe		853	-	4 3	-	-	-
Pipe only		179	-	3 6	-	-	-
Former smokers		543	652	1 2	1 7	-	-
Born 1926-45							
Nonsmokers		2831	4875	2 9	4 1	-	-
Present cpts	< 7	339	815	1 7	1 2	-	-
	8-15	743	1761	3 6	2 4	-	-
	>16	577	540	4 6	4 2	-	-
Cigarettes and pipe		1130	-	3 8	-	-	-
Pipe only		676	-	2 1	-	-	-
Former smokers		1346	1207	1 3	1 4	-	-
Born 1911-25							
Nonsmokers		1456	4207	3 5	4 5	0 7	0 7
Present cpts	<10	1041	975	3 6*	2 7*	5 1*	4 9*
	>10	635	285	6 4	5 5	13 4	15 0
Former cpts	<10	324	242	1 3	0 8	1 7	0 6
	>10	200	38	1 0	(1 2)	1 4	-
Born 1901-10							
Nonsmokers		741	2909	6 7	6 0	2 5	1 7
Present cpts	<10	457	239	2 2*	2 7*	2 6*	3 8*
	>10	189	67	5 1	5 2	6 3	7 0
Former cpts	<10	178	70	1 0	1 7	0 8	2 6
	>10	90	8	0 6	(1 5)	0 8	(5 3)
AMERICAN REGISTRY							
Born 1917-27							
Nonsmokers		865	-	4 3	-	2 2	-
Present cpts	<10	297	-	1 5	-	1 5	-
	11-30	1435	-	3 6	-	4 4	-
	>30	566	-	6 4	-	9 1	-
Cigar and pipe only		255	-	1 7	-	2 0	-
Former smokers		889	-	0 7	-	3 8	-

1) Not studied

Smoking in relation to coronary heart disease was investigated clinically in the Lundman study (1966). There was no evidence of excess morbidity from overt coronary heart disease in the smoking twins; no of any differences between smokers and nonsmokers regarding post exercise ST changes indicating coronary insufficiency. In table 7.3 page 86 are given the number of concordant and discordant pairs with respect to coronary heart disease and in relation to smoking exposure. Coronary heart disease was considered as diagnosed if the person had clinical history of previous myocardial infarction or coronary insufficiency (ST ≥ 5 mm horizontal or downward sloping). There were 8 pairs 4 MZ and 4 DZ with concordant coronary heart disease findings and 29 pairs 9 MZ and 20 DZ with discordant coronary heart disease findings. In 6 of the MZ pairs it was the nonsmoking partner who had the positive findings and in 3 it was the smoking partner. For the DZ pairs the corresponding figures were 11 and 9 respectively.

Since both high blood pressure and high serum cholesterol and tri glyceride levels are associated risk factors for coronary heart disease hypothesis could be that one should find higher lipid values and higher blood pressure among the smoking twins in the smoking discordant pairs. However the analysis in the present study showed only weak association between smoking and the serum lipids cholesterol and tri glycerides; their levels were slightly lower in the smoking twins. The smokers had lower mean weight by around 3 kg.

Systolic and diastolic blood pressure showed consistent tendency to be lower in the cigarette exposed twins in both the monozygotic and dizygotic groups. The diastolic blood pressure in relation to smoking is given in figure 7.4. It is evident that prolonged cigarette smoking was not associated with persistent elevation of the blood pressure.

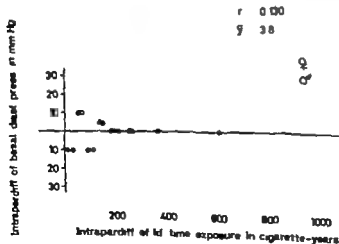


Figure 7.4 Correlation between Intrapair Differences for Smoking Exposure and Basal Diastolic Pressure in 37 Smoking Discordant Monozygotic Pairs (Correlation Coefficient; y Mean of Intrapair Difference) (from Lundman 1966)

compiled by the former National Air Pollution Control Administration in Research Triangle Park USA. One of these indices took into account the number of years the subject had lived in certain areas and the average air pollution measured. Another index considered only the subjects own reports as to how many years they had lived in downtown suburban or rural areas. Only the last mentioned index related to the prevalence of the respiratory symptoms. This relation was found in the A-series analyses but not in the B-series. Due to low numbers the implication of the negative finding was considered limited.

In the Lundman study (1966) based on 92 monozygotic and 104 dizygotic same-sexed smoking discordant pairs aged 37-77 years the degree of uneven ventilation according to a nitrogen washout delay method correlated well with cigarette consumption. The forced vital capacity (FVC) and forced expiratory volume during 1 sec (FEV_{1.0}) were significantly lower for smokers in a manner correlated with the amount of cigarette consumption. The differences found were as high as or higher than had previously been reported between smokers and nonsmokers. The lung function was affected to the same extent in males and females. These results were found among both monozygotic and dizygotic twins.

7 2 3 Cardiovascular findings

Former reports from the twin registries in regard to the mailed questionnaire studies have mainly focused on the symptoms operationally defined as angina pectoris (cf section 4 5 1). From the A-series analyses male smokers appeared to have a significant hypermorbidity ratio of 1.6 while female smokers did not show any significant hypermorbidity. In the American study current smokers of 30 or more cigarettes a day showed a hypermorbidity of 2.3 and former smokers a hypermorbidity of 1.9 both figures statistically significant. The B series analysis on the Swedish twins did not show any hypermorbidity ratio in any zygosity group while that on the American series showed a significant hypermorbidity ratio of 1.6 in the dizygotic twins. No hypermorbidity was seen in the monozygotic twins.

Table 7 3 Number of Concordant and Discordant pairs by Zygosity with Respect to Coronary Heart Disease in Relation to Smoking Exposure (from Lundman 1966)

		Exposed			DX		
		+	-	total	+	-	total
Not exposed	+	4	6	10	4	11	15
	-	3	24	27	9	38	47
	total	7	30	37	13	49	62

+ Coronary heart disease (see text)

- No coronary heart disease

Psycho-social item compared to partner is

	More	Equal	Less
Conflicts at work	6	16	2
Ambition at work	10	11	3
Overtime at work	8	12	4
Dedication to work	11	9	4

$p < 0.05$

Infarction No infarction

Figure 7.5 Intrapair Difference for Psycho-social Items in 24 Infarction Discordant Pairs (Infarction Partner Compared to "Healthy" Partner)
(Condensed from Liljafors 1970)

Coronary heart disease in male twins was studied from another point of departure by Liljefors (1970). Twin pairs were selected on the basis of positive replies on the questionnaire from the one or both twins in regard to angina pectoris (for one or both members in the pairs). Among 91 pairs 89 could be classified with regard to the presence or absence of coronary heart disease. Thirty-five pairs were found to be coronary heart disease discordant. 23 pairs coronary heart disease concordant and 31 pairs healthy concordant. Of the coronary heart disease discordant pairs 27 were discordant with respect to myocardial infarction but in 10 of these pairs the partner had angina pectoris or other signs of coronary heart disease. The smoking habit in the Liljefors study was found to be almost the same among the 35 coronary heart disease discordant pairs: 77 percent of the diseased and 74 percent of the healthy twins being present or former smokers. When the coronary heart disease concordant pairs were compared with healthy concordant pairs significantly more of the coronary heart disease pairs were found to be smokers: 89 against 74 percent.

On the basis of a personal interview the social background and psycho-social strain during adult life of 24 infarction discordant pairs 20-40 years of age were classified according to a point scale. The most interesting results were found regarding psycho-social strain at work in matters such as conflicts, ambition and overtime. The two latter factors were combined and this score was added to the score dedication to work. As is seen in figure 7.5 consistently more of the twins with a myocardial infarction had some psycho-social strain compared to their healthy partners. For ambition the difference reached statistical significance.

The importance of drinking and several other factors for the assessment of the relationship between smoking and angina pectoris was analyzed jointly on the Swedish and American twin series (Hrubec, Cederlöf and Friberg 1976). The rationale of the analysis was to form factor discordant subsamples of twin pairs with concordant smoking habits. Factors considered included drinking, low physical activity, frequent change of employer and occupational strain. All of these factors showed in a multiple correlation analysis significant partial correlations with angina pectoris in the A-series analysis. In the B-series analysis however only drinking turned out to be significantly related to the symptom in question.

Finally in the Floderus (1974) study angina pectoris was related to another disease entity: nervous diseases and a series of background variables indicating psycho-social strains (cf section 4.4). It was found that angina pectoris in males was 1.5 times and in females 2 times more prevalent among those who complained of nervous disease. High ratios were also found for almost all of the psycho-social items. For example angina pectoris was 1.5-1.7 times as common among those who had financial problems and 2.3 times as common among those who were not able to set aside work. Further marriage difficulties gave ratios of 1.5-1.9, shortage of time 1.4, restlessness 2.0 and sleeping difficulties 1.9. By studying smokers and nonsmokers separately only minor differences were found between the groups in regard to the risk ratio for angina pectoris and if anything the ratios were higher among nonsmokers. However several of the psycho-social items were related to smoking. On the basis of B-series analysis on item discordant monozygotic and dizygotic pairs Floderus concludes that the psycho-social discord items were found to discriminate subjects with respect to angina pectoris also when the influences of genetic and

Table 7 6 Continuation

		Non- Born smokers	Cigarettes only			Ciga- rettes and pipe	Pipe only	Former smokers
		Prevalence Rate	<7	8-15	>15			
			Ratio			Ratio		
Longlasting illness								
Males	1956-58	9 5	0 6	1 1	0 0	2 6	0 0	1 1
	1946-55	11 4	0 8	1 0	1 3	1 1	1 2	1 1
	1926-45	16 5	1 2	1 2	1 2	1 3	1 0	1 2
Females	1956-58	7 9	1 6	2 4	(3 2)	-		1 7
	1946-55	10 4	0 9	1 1	1 8			1 2
	1926-45	15 9	1 1	1 2	1 6			1 4
Sick leave > 3 months in row								
Males	1956-58	8 9	3 3	1 7	0 0	4 2	0 0	1 1
	1946-55	4 4	1 3	1 7	2 2	1 7	1 2	1 6
	1926-45	12 2	1 1	1 6	1 6	1 8	1 2	1 2
Females	1956-58	0 4	2 5	0 0	(20 8)			0 0
	1946-55	3 3	1 5	2 1	3 7			1 4
	1926-45	11 0	1 2	1 6	2 4			1 5

7 2 4 Selected disease entities other than respiratory and cardiovascular

In the two twin registries in Sweden some questions were also asked about morbidity other than respiratory and cardiovascular

The questionnaire mailed to the new twin registry asked for information about series of symptoms as well as long-lasting illness and sick leave for more than and equal to 3 months in a row Table 7 6 presents data in the A-series fashion

It appears from the table that every item listed shows a significant hypermorbidity in one or more of the subgroups The table also contains individual who were between 15 and 17 years of age at time of observation and several diseases such as stomach disorders back disorder impaired hearing and long-lasting illness were already significantly increased among such subjects who smoked less than 8 cigarettes a day Stomach and back disorders are clearly related to number of cigarettes smoked even in this youngest age group

Without exception all items show dose related hypermorbidity ratios in the older age groups for both males and females Cigarette and pipe smoking and pipe only smoking also show significant excess morbidity for most of the symptoms in the youngest age group as well though the numbers are not large enough to make the ratios significant.

Table 7 6 Selected Data on Diseases by Sex and Age; Age-adjusted Morbidity Ratios in Smoking Groups in Relation to Prevalence Rates among Nonsmokers; New Swedish Twin Registry A-series¹

		Non- smokers	Cigarettes only			Ciga- rettes and pipe	Pipe only	Former smokers
	Born		<7	8-15	>16			
		Prevalence Rate	Ratio			Ratio		
<hr/>								
Stomach disorders								
Males	1956-58	4.1	1.1	1.9	(3.5)	2.8	(1.4)	1.4
	1946-55	9.2	1.5	1.7	2.3	2.0	1.5	1.3
	1926-45	12.2	1.7	1.7	2.3	2.2	1.4	1.7
Females	1956-58	7.7	1.6	2.7	(2.2)	-	-	1.5
	1946-55	17.2	1.3	1.6	2.5	-	-	1.4
	1926-45	19.4	1.1	1.6	2.1	-	-	1.3
Back disorders								
Males	1956-58	4.1	2.9	3.0	(10.5)	4.7	(4.1)	2.4
	1946-55	9.3	1.3	1.8	2.5	1.9	1.4	1.4
	1926-45	16.7	1.2	1.3	1.4	1.5	1.4	1.1
Females	1956-58	4.4	2.5	3.6	(1.9)	-	-	2.2
	1946-55	6.9	1.3	2.0	3.2	-	-	1.3
	1926-45	13.4	1.1	1.5	1.7	-	-	1.4
Decreased hearing								
Males	1956-58	4.1	0.4	2.2	0.0	1.9	(1.4)	2.1
	1946-55	6.2	1.7	1.3	1.2	1.5	1.6	0.9
	1926-45	10.2	1.2	1.3	1.2	1.4	1.5	1.4
Females	1956-58	2.6	2.8	3.3	(6.4)	-	-	3.3
	1946-55	3.1	0.9	1.4	2.7	-	-	1.1
	1926-45	4.8	1.2	1.0	1.6	-	-	1.4
Migrain								
Males	1956-58	1.3	2.3	1.2	0.0	1.0	0.0	0.8
	1946-55	1.2	1.7	1.8	2.9	1.6	0.7	1.0
	1926-45	2.8	0.7	1.3	1.5	1.5	1.3	0.9
Females	1956-58	1.4	1.7	1.4	(5.9)	-	-	0.7
	1946-55	4.4	1.2	1.3	2.1	-	-	1.3
	1926-45	8.3	0.7	1.0	1.4	-	-	1.2
Asthma								
Males	1956-58	18.2	0.9	0.9	(1.6)	1.1	(1.5)	0.9
	1946-55	14.1	1.0	0.9	1.1	1.1	1.1	1.3
	1926-45	14.2	0.8	0.9	0.8	1.0	0.9	1.0
Females	1956-58	17.9	1.1	1.4	(3.3)	-	-	1.3
	1946-55	17.1	1.0	1.1	1.4	-	-	1.2
	1926-45	17.1	1.0	1.0	1.3	-	-	1.2

1) For numbers at risk see table 6 1

continuation

Tabl 7 E Continuation

		Cigarettes only			Ciga- rettes	Pipe	Former	
Born	Non- smokers	<7	8-15	>16	and pipe	only	smokers	
Prevalence Rate		Ratio			Ratio			
<hr/>								
Longlasting illness								
Males	1956-58	9.5	0.6	1.1	0.0	2.6	0.0	1.1
	1946-55	11.4	0.8	1.0	1.3	1.1	1.2	1.1
	1926-45	16.5	1.2	1.2	1.2	1.3	1.0	1.2
Female	1956-58	7.9	1.6	2.4	(3.2)			1.7
	1946-55	10.4	0.9	1.1	1.8		-	1.2
	1926-45	15.9	1.1	1.2	1.6		-	1.4
Sick leave > 3 months in row								
Males	1956-58	0.9	3.3	1.7	0.0	4.2	0.0	1.1
	1946-55	4.4	1.3	1.7	2.2	1.7	1.2	1.6
	1926-45	12.2	1.1	1.6	1.6	1.8	1.2	1.2
Female	1956-58	0.4	2.5	0.0	(20.0)			0.0
	1946-55	3.3	1.5	2.1	3.7		-	1.4
	1926-45	11.0	1.2	1.6	2.4			1.5

7.2.4 Selected disease entities other than respiratory and cardiovascular

In the two twin registries in Sweden some questions were also asked about morbidity other than respiratory and cardiovascular.

The questionnaire mailed to the new twin registry asked for information about series of symptoms as well as long-lasting illness and sick leave for more than and equal to 3 months in row. Table 7.6 presents data in the A-series fashion.

It appears from the table that every item listed shows a significant hypermorbidity in one or more of the subgroups. The table also contains individuals who were between 15 and 17 years of age at time of observation and several diseases such as stomach disorders, back disorders, impaired hearing, and long-lasting illnesses were already significantly increased among such subjects who smoked less than 8 cigarettes a day. Stomach and back disorders are clearly related to number of cigarettes smoked even in this youngest age group.

Without exception all items show dose related hypermorbidity ratios in the older age groups of both males and females. Cigarette and pipe smoking and pipe only smoking also show significant excess morbidity for most of the symptoms in the youngest age group as well though the numbers are not large enough to make the ratios significant.

Table 7 6 Selected Data on Diseases by Sex and Age; Age-adjusted Morbidity Ratios in Smoking Groups in Relation to Prevalence Rates among Nonsmokers; New Swedish Twin Registry A-series

	Born	Non- smokers	Cigarettes only			Ciga- rettes and pipe	Pipe only	Former smokers
			<7	8-15	>16			
			Prevalence Rate	Ratio				
<hr/>								
Stomach disorders								
Males	1956-58	4 1	1 1	1 9	(3 5)	2 8	(1 4)	1 4
	1946-55	9 2	1 5	1 7	2 3	2 0	1 5	1 3
	1926-45	12 2	1 7	1 7	2 3	2 2	1 6	1 7
Females	1956-58	7 7	1 6	2 7	(2 2)	-	-	1 5
	1946-55	17 2	1 3	1 6	2 5	-	-	1 4
	1926-45	19 4	1 1	1 6	2 1	-	-	1 3
Back disorders								
Males	1956-58	4 1	2 9	3 0	(10 5)	4 7	(4 1)	2 4
	1946-55	9 3	1 3	1 8	2 5	1 9	1 4	1 4
	1926-45	16 7	1 2	1 3	1 4	1 5	1 4	1 1
Females	1956-58	4 4	2 5	3 6	(1 9)	-	-	2 2
	1946-55	6 9	1 3	2 0	3 2	-	-	1 3
	1926-45	13 4	1 1	1 5	1 7	-	-	1 4
Decreased hearing								
Males	1956-58	4 1	0 4	2 2	0 0	1 9	(1 4)	2 1
	1946-55	6 2	1 7	1 3	1 2	1 5	1 6	0 9
	1926-45	10 2	1 2	1 3	1 2	1 4	1 5	1 4
Females	1956-58	2 6	2 8	3 3	(6 4)	-	-	3 3
	1946-55	3 1	0 9	1 4	2 7	-	-	1 1
	1926-45	4 8	1 2	1 0	1 6	-	-	1 4
Migrain								
Males	1956-58	1 3	2 3	1 2	0 0	1 0	0 0	0 8
	1946-55	1 2	1 7	1 8	2 9	1 6	0 7	1 0
	1926-45	2 8	0 7	1 3	1 5	1 5	1 3	0 9
Females	1956-58	1 4	1 7	1 4	(5 9)	-	-	0 7
	1946-55	4 4	1 2	1 3	2 1	-	-	1 3
	1926-45	8 3	0 7	1 0	1 4	-	-	1 2
Asthma								
Males	1956-58	18 2	0 9	0 9	(1 6)	1 1	(1 5)	0 9
	1946-55	14 1	1 0	0 9	1 1	1 1	1 1	1 3
	1926-45	14 2	0 8	0 9	0 8	1 0	0 9	1 0
Females	1956-58	17 9	1 1	1 4	(3 3)	-	-	1 3
	1946-55	17 1	1 0	1 1	1 4	-	-	1 2
	1926-45	17 1	1 0	1 0	1 3	-	-	1 2

1) For numbers at risk see table 6 1

continuation

Tabl 7 6 Continuation

	born	Non-smoker	Cigarette only			Cigarettes and pipe	Pipe only	Former smokers
			<7	8-15	>16			
			Prevalence Rate	Ratio				
<hr/>								
Longlasting illness								
Males	1956-58	9 3	0 6	1 1	0 0	2 6	0 0	1 1
	1946-55	11 4	0 8	1 0	1 3	1 1	1 2	1 1
	1926-45	16 5	1 2	1 2	1 2	1 3	1 0	1 2
Females	1956-58	7 9	1 6	2 4	(3 2)	-		1 7
	1946-55	10 4	0 9	1 1	1 8			1 2
	1926-45	15 9	1 1	1 2	1 6			1 4
Sick leave 3 months in row								
Males	1956-58	8 9	3 3	1 7	0 0	4 2	0 0	1 1
	1946-55	4 4	1 3	1 7	2 2	1 7	1 2	1 6
	1926-45	12 2	1 1	1 6	1 6	1 8	1 2	1 2
Females	1956-58	0 4	2 5	0 0	(20 8)			0 0
	1946-55	3 3	1 5	2 1	3 7			1 4
	1926-45	11 0	1 2	1 6	2 4			1 5

7 2 4 Selected disease entities other than respiratory and cardiovascular

In the two twin registries in Sweden some questions were also asked about morbidity other than respiratory and cardiovascular

The questionnaire mailed to the new twin registry asked for information about series of symptoms as well as long-lasting illness and sick leave for more than and equal to 3 months in a row. Tabl 7 6 presents data in the A-series fashion.

It appears from the tabl that every item listed shows a significant hypermorbidity in one or more of the subgroups. The table also contains individuals who were between 15 and 17 years of age at time of observation and several diseases such as stomach disorders, back disorders, impaired hearing and long-lasting illness were already significantly increased among such subjects who smoked less than 8 cigarettes a day. Stomach and back disorders are clearly related to number of cigarettes smoked even in this youngest age group.

Without exception all items show dose related hypermorbidity since in the older age groups of both males and females cigarette and pipe smoking and pipe only smoking also show significant excess morbidity for most of the symptoms in the youngest age group as well though the numbers are not large enough to make the ratios significant.

Table 7 6 Selected Data on Diseases by Sex and Age; Age-adjusted Morbidity Ratios in Smoking Groups in Relation to Prevalence Rates among Nonsmokers; New Swedish Twin Registry A-series¹

	Born	Non smokers	Cigarettes only			Ciga- rettes and pipe	Pipe Former only smokers	
		Prevalence Rate	<7	8-15	≥16			Ratio
<hr/>								
Stomach disorders								
Males	1956-58	4 1	1 1	1 9	(3 5)	2 8	(1 4)	1 4
	1946-55	9 2	1 5	1 7	2 3	2 0	1 5	1 3
	1926-45	12 2	1 7	1 7	2 3	2 2	1 6	1 7
Females	1956-58	7 7	1 6	2 7	(2 2)	-	-	1 5
	1946-55	17 2	1 3	1 6	2 5			1 4
	1926-45	19 4	1 1	1 6	2 1	-		1 3
Back disorders								
Males	1956-58	4 1	2 9	3 0	(10 5)	4 7	(4 1)	2 4
	1946-55	9 3	1 3	1 8	2 5	1 9	1 4	1 4
	1926-45	16 7	1 2	1 3	1 4	1 5	1 4	1 1
Females	1956-58	4 4	2 5	3 6	(1 9)	-		2 2
	1946-55	6 9	1 3	2 0	3 2	-		1 3
	1926-45	13 4	1 1	1 5	1 7		-	1 4
Decreased hearing								
Males	1956-58	4 1	0 4	2 2	0 0	1 9	(1 4)	2 1
	1946-55	6 2	1 7	1 3	1 2	1 5	1 6	0 9
	1926-45	10 2	1 2	1 3	1 2	1 4	1 5	1 4
Females	1956-58	2 6	2 8	3 3	(6 4)			3 3
	1946-55	3 1	0 9	1 4	2 7		-	1 1
	1926-45	4 8	1 2	1 0	1 6			1 4
Migrain								
Males	1956-58	1 3	2 3	1 2	0 0	1 0	0 0	0 8
	1946-55	1 2	1 7	1 8	2 9	1 6	0 7	1 0
	1926-45	2 8	0 7	1 3	1 5	1 5	1 3	0 9
Females	1956-58	1 4	1 7	1 4	(5 9)	-		0 7
	1946-55	4 4	1 2	1 3	2 1			1 3
	1926-45	8 3	0 7	1 0	1 4			1 2
Asthma								
Male	1956-58	18 2	0 9	0 9	(1 6)	1 1	(1 5)	0 9
	1946-55	14 1	1 0	0 9	1 1	1 1	1 1	1 3
	1926-45	14 2	0 8	0 9	0 8	1 0	0 9	1 0
Female	1956-58	17 9	1 1	1 4	(3 3)			1 3
	1946-55	17 1	1 0	1 1	1 4			1 2
	1926-45	17 1	1 0	1 0	1 3		-	1 2

1) For numbers at risk see table 6 1

continuation

Tabl 7 8 Selected Data on Diseases; Sex and Age Standardized Ratios of Observed Over Expected Prevalence Rates Among Nonsmokers Grouped by Smoking Status of Co-Twin; New Swedish Twin Registry MET-series

			Non- Smokers	Present Cigarette Smokers	All Present Smokers	Former Smokers
No t risk	ME		1001	140	423	270
	DE		2236	1010	1307	544
Stomach disorders	ME		0 97	1 33	1 35	1 42
	DE		0 94	1 17	1 15	1 06
Back disorders	ME		0 86	1 35	1 24	1 41
	DE		0 96	1 06	1 05	0 90
Decreased hearing	ME		1 02	1 20	1 24	1 11
	DE		0 99	1 18	1 04	1 00
Migrain	ME		0 90	2 64	2 05	0 76
	DE		0 91	1 10	1 14	1 19
Asthma	ME		0 97	1 24	1 20	1 32
	DE		1 01	0 87	0 90	1 07
Longlasting illness	ME		0 99	1 02	1 09	1 54
	DE		0 99	0 93	0 96	1 27
Sick leave 3 months in row	ME		0 92	1 55	1 53	0 96
	DE		1 00	1 03	1 13	1 24

Table 7 7 Selected Data on Diseases; Sex and Age Standardized Ratios of Observed Over Expected Prevalence Rates Among Nonsmokers Grouped by Smoking Status of Co-Twin; New Swedish Twin Registry NET-series

		Non-Smokers	Present Cigarette Smokers	All Present Smokers	Former Smokers
No at risk	ME	1881	340	423	270
	DE	2236	1010	1307	544
Cough	ME	0 88	2 71	1 72	1 28
	DE	1 00	1 11	1 07	0 76
Pains in the chest	ME	0 97	1 62	1 32	1 24
	DE	0 94	1 04	0 95	1 34
Shortness of breath	ME	0 94	1 25	1 24	1 24
	DE	0 93	1 06	1 07	1 05

Former smokers also display increased hypermorbidity ratios for all items except possibly migraine. The ratios are of about the same order of magnitude as in the group of present cigarette smokers smoking 7 cigarettes or less a day.

7 2 5 Symptoms among nonsmoking partners of smoking co-twins

In section 6 3 it was shown that several items such as drinking and indications of psycho-social discord were more prevalent among nonsmoking partners of presently smoking co-twins than could be expected from prevalence rates in the total group of nonsmokers. This section will deal with the same type of analysis with respect to the symptoms reported from the New Registry in foregoing sections.

Table 7 7 displays sex- and age standardized prevalence ratios of cough, pains in the chest and shortness of breath among nonsmoking partners grouped according to the smoking status of their co-twins. It appears that all three symptoms show significantly increased ratios in the monozygotic twins with presently smoking partners. This effect is however not seen among dizygotes except for pains in the chest in twins with formerly smoking partners.

Table 7 8 comprises data on other symptoms corresponding to the data in table 7 6. It is again seen that all items show with one single exception hypermorbidity ratios that are higher and very often significantly so among the monozygotes. Among the highest ratios are migraine and sick leave more than and equal to 3 months in a row.

Table 7 B Selected Data on Diseases; Sex and Age Standardized Ratios of Observed Over Expected Prevalence Rates Among Nonsmokers Grouped by Smoking Status of Co-Twin; New Swedish Twin Registry MET-series

		Non- Smokers	Present Cigarette Smoker	All Present Smokers	Former Smokers
No t risk	ME	1881	340	423	270
	DE	2236	1010	1307	544
Stomach disorders	ME	0 97	1 33	1 35	1 42
	DE	0 94	1 17	1 15	1 06
Back disorders	ME	0 86	1 35	1 34	1 41
	DE	0 96	1 06	1 05	0 90
Decreased hearing	ME	1 02	1 20	1 24	1 01
	DE	0 99	1 18	1 04	1 00
Migrain	ME	0 90	2 64	2 0	0 76
	DE	0 91	1 10	1 14	1 19
Asthma	ME	0 97	1 24	1 20	1 32
	DE	1 01	0 87	0 90	1 07
Longlasting illness	ME	0 99	1 02	1 09	1 34
	DE	0 99	0 93	0 96	1 27
Sick leave >3 months in row	ME	0 92	1 55	1 53	0 96
	DE	1 00	1 03	1 03	1 24

7 3 1 Introductory remarks

The follow-up of mortality in the old Swedish twin registry has been going on since the start of the registry in 1961. The first results were reported in 1970 and a second publication appeared in 1973. The last mentioned report included all deaths that had occurred before July 1 1972. The following tables will present data on deaths accumulated up to and including June 1975. The presentation by and large will follow the main principles laid down in the 1973 report. Thus A-series analyses are first presented giving accumulated rate of deaths in nonsmokers and hypermortality ratios by age and sex in different smoking categories. All prevalence rates and accordingly the hypermortality ratios are age adjusted on the basis of 5-year age intervals. The outcome of the A analyses will not be commented upon if they do not notably deviate from what is generally found in studies on unrelated smokers and nonsmokers.

As in the earlier reports the B-series analyses focus on the concept of first deaths. This is necessary because of the longitudinal approach; the working hypothesis is that a smoker in a smoking discordant pair will die on an average before his nonsmoking partner. Thus for pairs with two deceased twins only the first deceased twin is included in the subsequent analysis. However in order not to suppress information that may interest the reader the second deaths are included in the tables as subscripts.

Table 7 9 Age-adjusted Mortality Rates among Nonsmokers and Mortality Ratios by Sex Age and Smoking Groups; Old Swedish Twin Registry A-series

		Present Smokers				Former Smokers		
	Born	Non smokers	<10 cigs	>10 cigs	Cigar/ pipe	<10 cigs	>10 cigs	Cigar/ pipe
No at Risk								
Males	1911-25	1707	1249	793	890	384	239	157
	1901-10	881	537	231	472	212	99	184
Females	1911-25	4770	1111	322	-	286	44	-
	1901-10	2824	285	75	-	76	9	-
		Rate		Ratios			Ratios	
Mortality								
Males	1911-25	4.7	1.7	2.0	1.3	1.1	1.6	1.0
	1901-10	16.3	1.2	2.2	1.3	1.1	1.8	0.8
Females	1911-25	3.6	1.5	2.0	-	1.3	(2.2)	
	1901-10	13.2	1.1	1.8	-	0.9	(0.6)	

- 1) The Department of Medicine of the Karolinska Institute at the Serafiner Hospital Stockholm
- 2) The Department of Environmental Hygiene the Karolinska Institute Stockholm

The detailed classification of smoking discordance presented in Chapter 4 is the same as in previous reports yielding two groups for analyses. One group contains nonsmokers versus cigarette smokers¹. The other group consists of the same pair as the before mentioned group plus a number of pairs where both members smoke cigarettes but are discordant in regard to quantity. The last mentioned pooled groups are designated as "pooled low group" versus "pooled high group"².

The B-series tables include present smoker and former smokers (top of table) as well as present smokers only (bottom of table).

7.3.2 Gross mortality

Age adjusted mortality rates are presented in the A series fashion in table 7.9 by sex, age and smoking groups.

As the first step in the B-series analyses mortality data are given as incidences during three successive 5-year intervals since the beginning of the study in 1961. Table 7.10 shows the incidence by sex, zygosity and year of death in the pooled low and pooled high groups. Only the total cohort is presented as further breakdown would render the numbers too low.

Table 7.10 Mortality by Sex, Zygosity and Year of Death and the Total Cohort of Smoking Discordance Groups; Old Swedish Twin Registry, Age Group 1901-25, First Deaths Only, B-series

DIZYGOTES					MONOZYGOTES		
Year of Death	Person Year of Observation	Pooled Low Group	Pooled High Group		Person Years of Observation	Pooled Low Group	Pooled High Group
PRESENT AND FORMER SMOKERS							
Males	1961-65	3502	9	16	1224	8	4
	1966-70	3318	17	30	1129	10	10
	1971-75	2749	28	36	933	6	13
Females	1961-65	3082	7	12	1622	3	4
	1966-70	3737	18	22	1585	5	5
	1971-75	3172	19	26	1343	14	17
PRESENT SMOKERS ONLY							
Males	1961-65	2416	6	7	831	6	3
	1966-70	2299	13	22	773	6	7
	1971-75	1896	18	27	629	3	9
Females	1961-65	3084	6	11	1251	2	1
	1966-70	2948	15	16	1230	3	5
	1971-75	2515	17	21	1037	12	16

1) The cigarette smoker may also smoke pipe and/or cigar

2) The "high smoker" may also smoke pipe and/or cigars

3) June 1975

Table 7 11 Mortality¹ by Sex Age Zygosity and Smoking Discordance Groups; Old Swedish Twin Registry B series

		DIZYGOTES			MONOZYGOTES		
Born		No of Pairs	Non-smokers	Smokers	No of Pairs	Non-smokers	Smokers
PRESENT AND FORMER SMOKERS							
Nonsmokers versus Smokers							
Males	1911-25	364	18	21 ₂	115	3 ₂	8
	1901-10	157	19 ₅	34 ₁	46	7 ₁	7
	1901-25	521	37 ₅	55 ₃	161	10 ₁	15 ₄
Females	1911-25	510	18 ₃	29 ₂	213	12	13
	1901-10	174	25 ₃	23 ₁	59	6	9 ₂
	1901-25	684	43 ₆	52 ₃	272	18 ₁	22 ₃
Pooled Low versus Pooled High Group		No of Pairs	Low	High	No of Pairs	Low	High
Males	1911-25	501	26 ₂	34 ₂	177	9	14
	1901-10	205	28 ₆	48 ₄	69	15 ₁	13
	1901-25	706	54 ₈	82 ₆	246	24 ₄	27 ₈
Females	1911-25	588	18 ₃	31 ₂	262	15	17 ₂
	1901-10	193	26 ₃	29 ₁	64	7 ₁	9 ₁
	1901-25	781	44 ₆	60 ₃	326	22 ₁	26 ₃
PRESENT SMOKERS ONLY							
Nonsmokers versus Smokers							
Males	1911-25	274	13	19 ₁	77	2 ₁	7
	1901-10	101	14 ₅	21 ₁	34	5 ₁	4 ₃
	1901-25	375	27 ₅	40 ₂	111	7 ₁	11 ₃
Females	1911-25	421	16 ₃	23 ₂	167	10	11 ₁
	1901-10	131	21 ₂	18 ₁	48	5 ₁	7 ₁
	1901-25	552	37 ₅	41 ₃	215	15 ₁	18 ₂
Pooled Low versus Pooled High Group		No of Pairs	Low	High	No of Pairs	Low	High
Males	1911-25	356	16 ₁	28 ₁	123	6 ₁	12
	1901-10	130	21 ₅	28 ₄	44	9 ₂	7 ₅
	1901-25	486	37 ₆	56 ₅	167	15 ₃	19 ₅
Females	1911-25	477	16 ₃	25 ₂	199	11	15 ₁
	1901-10	144	22 ₂	23 ₁	52	6 ₁	7 ₁
	1901-25	621	38 ₅	48 ₃	251	17 ₁	22 ₂

1) In pairs in which both members have died first deceased member has been recorded in proper smoking status group while partner is shown in subscript number Total number of deaths can be obtained by adding subscript figures to main figures As an example among 364 nonsmoking smoking dizygotic male twin pairs belonging to 1911-25 age group 18 first deaths have occurred among nonsmokers and 21 first deaths among smokers Two more deaths were found among smokers but this occurred

It is apparent from table 7 10 that the incidence as far as the dizygotes are concerned has been consistently higher for the pooled high group than for the pooled low group throughout all three 5-year periods. This is true for both males and females and also when former smokers are excluded. In the monozygotic groups the numbers are low. No consistent difference can be noted between the pooled low group and the pooled high group during the first 10 years of observation. During the last 5 year however higher although not significantly higher incidence has been noted in the pooled high group for both sexes. When males and females are taken together the monozygotic incidence ratios during this last period of observation are 1.5 for present and former smokers and 1.7 for present smokers only. These ratios are about the same as those for the dizygotes which are 1.3 and 1.4 respectively.

In table 7 11 the total accumulated mortality up to and including June 1975 is presented by sex, age and zygosity in the two smoking discordance groups. The following comments relate primarily to the total cohort born 1901-1925 since the numbers are low when the age groups are taken separately.

Among the dizygotes the differences between nonsmokers and smokers and between the two pooled exposure groups are apparent for both males and females. For the total cohort of present and former smokers born 1901-1925 the hypermortality ratios are about 1.5 for males and about 1.2-1.4 for females. These ratios are significant for males. The monozygotic groups also show excess deaths among the exposed. The numbers are low however and the observed differences do not reach statistical significance. About the same excess is seen irrespective of whether former smokers are excluded or not. The hypermortality in the exposed dizygotes is found mainly in the younger age-group.

7 3 3 Selected cause-specific mortality

Just as in the earlier reports from the Swedish Twin Registry cause-specific mortality has here been classified for the diagnoses coronary heart disease, cardiovascular disease, cancer of the lung, other forms of cancer, suicides and accidents. These entities together with other causes are presented in the A-series fashion in table 7 12, page 96 for males and females respectively. The results are not age adjusted.

Coronary heart disease In the B-series analyses (table 7 13, page 99) because of small numbers only the pooled low group and the pooled high group are shown. In the dizygotic series both males and females show mortality rates that are about twice as high in the pooled high group as compared to the pooled low group, namely 25 as against 13 in males and 11 as against 5 in the females. Among the male monozygotes 10 cases are observed in the high group versus 8 in the low group. If former smokers are excluded male dizygotes show 18 deaths in the pooled high group and 7 in the pooled low group. Corresponding numbers for the monozygotes are 6 against 8. For females the numbers are 8.5 and 3.1 respectively.

Cancer of the lung In the B-series analysis (table 7 14, page 99) among the dizygotic males if former smokers included there were 9 lung cancer cases in the pooled high group as against 3 cases in the pooled low group. Among the male monozygotes two cases occurred in the pooled high group and one case in the pooled low group (cf section 8 3 3). Of the two cases in the dizygotic pooled low group one has occurred in a former smoker.

Table 7 12 Mortality Rates Among Nonsmokers and Mortality Ratios¹ from Selected Causes by Sex Age and Smoking Groups; Old Swedish Twin Register A-series²

		Present Smokers				Former Smokers		
Born		Non-smokers	<10	>10	Cigar/ pipe	<10	>10	Cigar pipe
			cyts	cyts		cyts	cyts	
		Prevalence Rate	Ratios		Ratios			
<hr/>								
Coronary Heart Disease								
Males	1911-25	1 1	2 1*	2 6	1 5	1 5	1 2	1 7
	1901-10	3 7	2 0	2 9*	2 5	1 8	3 8	1 5
Females	1911-25	0 3	1 0	1 0	-	1 3	(7 7)	
	1901-10	2 7	2 2	1 0	-	1 4	-	
Cerebrovascular Disease								
Males	1911-25	0 3	0 7	1 7	0 7	1 0	-	
	1901-10	1 2	1 6	1 4	2 1	1 2	0 8	1 3
Females	1911-25	0 3	1 3	5 3*	-	1 3	-	
	1901-10	1 7	0 6	0 8	-	-	-	
Cancer of the Lung								
Males	1911-25	0 06	5 5	12 9	7 7	-	-	21 7
	1901-10	0 23	6 6	24 8	5 6	2 1	4 4	-
Females	1911-25	0 04	-	7 4	-	-	-	-
	1901-10	0 11	6 6	12 6	-	-	-	-
Other Cancers								
Males	1911-25	1 2	0 5	1 3	1 1	1 5	1 8	0 5
	1901-10	3 6	1 2	1 3	1 2	1 8	1 4	0 6
Females	1911-25	1 8	1 3	1 1	-	1 1	(1 3)	-
	1901-10	4 5	0 7	1 8	-	1 8	-	-
Suicides								
Males	1911-25	0 4	2 5	2 8	1 8	0 8	2 0	-
	1901-10	0 7	0 3	1 9	0 9	0 7	1 4	-
Females	1911-25	0 2	2 0	8 0	-	-	(11 5)	-
	1901-10	0 2	-	-	-	-	-	-
Accidents								
Males	1911-25	0 5	1 4	2 6	1 2	0 6	2 6	-
	1901-10	1 5	0 4	1 7	0 7	0 6	1 3	-
Females	1911-25	0 3	0 7	2 0	-	-	(22 2)	-
	1901-10	0 5	1 4	-	-	-	-	-
Other Causes								
Males	1911-25	1 2	1 9	0 9	0 8	1 1	1 8	1 1
	1901-10	5 3	0 8	1 5	0 6	0 5	1 0	0 8
Females	1911-25	0 8	1 5	0 4	-	1 4	-	-
	1901-10	3 5	0 7	2 3	-	-	-	-

1) Not adjusted for age

2) For numbers at risk see table 7 9

Table 7 13 Mortality¹ from Coronary Heart Disease by Sex Age Zygosity and Smoking Discordance Groups; Old Swedish Twin Registry B-series

		DIZYGOTES			MONOZYGOTES		
	Born	No of Pairs	Pooled Low Group	Pooled High Group	No of Pairs	Pooled Low Group	Pooled High Group
PRESENT AND FORMER SMOKERS							
Males	1911 25	501	7	9 ₁	177	2	3
	1901 10	205	6 ₃	16 ₂	69	6 ₂	7 ₄
	1901 25	706	13 ₃	25 ₃	246	8 ₂	10 ₄
Females	1911 25	588	0	0	262	1	0
	1901 10	193	5 ₁	11 ₁	64	0	3 ₁
	1901 25	781	5 ₁	11 ₁	326	1	3 ₁
PRESENT SMOKERS ONLY							
Males	1911 25	356	2	8 ₁	123	2	3
	1901 10	130	5 ₃	10 ₂	44	4 ₂	3 ₂
	1901 25	486	7 ₃	18 ₃	167	6 ₂	6 ₂
Females	1911 25	477	0	0	199	1	0
	1901 10	144	5	6 ₁	52	0	3 ₁
	1901 25	621	5	8 ₁	251	1	3 ₁

Table 7 14 Mortality¹ from Cancer of the Lung by Sex Age Zygosity and Smoking Discordance Groups; Old Swedish Twin Registry B-series

		DIZYGOTES			MONOZYGOTES		
	Born	No of Pairs	Pooled Low Group	Pooled High Group	No of Pairs	Pooled Low Group	Pooled High Group
PRESENT AND FORMER SMOKERS							
Males	1911 25	501	0	2	177	0	0
	1901 10	205	2	7	69	1	2
	1901 25	706	2	9	246	1	2
Female	1911 25	588	0	0	262	8	0
	1901 10	193	0	1	64	1	0
	1901 25	781	0	1	326	1	0
PRESENT SMOKERS ONLY							
Males	1911 25	356	0	2	123	0	0
	1901 10	130	1	7	44	1	2
	1901 25	486	1	9	167	1	2
Females	1911 25	477	0	0	199	0	0
	1901 10	144	0	1	52	1	0
	1901 25	621	0	1	251	1	0

1) See footnote to table 7 11

Table 7 12 Mortality Rates Among Nonsmokers and Mortality Ratios¹ from Selected Causes by Sex Age and Smoking Groups; Old Swedish Twin Registry A-series²

		Present Smokers				Former Smokers		
Born	Non-smokers	<10	>10	Cigar/	Cigar/	<10	>10	Cigar/
		cgts	cgts	pipe		cgts	cgts	pipe
		Prevalence Rate	Ratios			Ratios		
<hr/>								
Coronary Heart Disease								
Males	1911-25	1 1	2 1	2 6	1 5	1 5	1 2	1 7
	1901-10	3 7	2 0	2 9	2 5	1 8	3 8	1 5
Females	1911-25	0 3	1 0	1 0	-	1 3	(7 7)	
	1901-10	2 7	2 2	1 0	-	1 4	-	
Cerebrovascular Disease								
Males	1911-25	0 3	0 7	1 7	0 7	1 0	-	-
	1901-10	1 2	1 6	1 4	2 1	1 2	0 8	1 3
Females	1911-25	0 3	1 3	5 3	-	1 3	-	
	1901-10	1 7	0 6	0 8	-	-	-	
Cancer of the Lung								
Males	1911-25	0 06	5 5	12 9	7 7	-	-	21 7
	1901-10	0 23	6 6*	24 8	5 6	2 1	4 4	-
Females	1911-25	0 04	-	7 4	-	-	-	
	1901-10	0 11	6 6	12 6	-	-	-	
Other Cancers								
Males	1911-25	1 2	0 5	1 3	1 1	1 5	1 8	0 5
	1901-10	3 6	1 2	1 3	1 2	1 8	1 4	0 6
Females	1911-25	1 8	1 3	1 1	-	1 1	(1 3)	-
	1901-10	4 5	0 7	1 8	-	1 8	-	
Suicides								
Males	1911-25	0 4	2 5	1 8	1 8	0 8	2 0	-
	1901-10	0 7	0 3	1 9	0 9	0 7	1 4	-
Females	1911-25	0 2	2 0	8 0	-	-	(11 5)	-
	1901-10	0 2	-	-	-	-	-	
Accidents								
Males	1911-25	0 5	1 4	2 6	1 2	0 6	2 6	
	1901-10	1 5	0 4	1 7	0 7	0 6	1 3	
Females	1911-25	0 3	0 7	2 0	-	-	-	
	1901-10	0 5	1 4	-	-	-	(22 2)	-
Other Causes								
Males	1911-25	1	1 9	0 9	0 8	1 1	1 8	1 1
	1901-10	5 3	0 8	1 5	0 6	0 5	1 0	0 8
Females	1911-25	0 8	1 5	0 4	-	1 4	-	-
	1901-10	3 5	0 7	2 3	-	-	-	

1) Not adjusted for age

2) For numbers at risk see table 7 9

Table 7 13 Mortality¹ from Coronary Heart Disease by Sex Age Zygosity and Smoking Discordance Groups; Old Swedish Twin Registry B-series

		DIZYGOTES			MONOZYGOTES		
	Born	No of Pairs	Pooled Low Group	Pooled High Group	No of Pairs	Pooled Low Group	Pooled High Group
PRESENT AND FORMER SMOKERS							
Males	1911 25	501	7	9 ₁	177	2	3
	1901 10	205	6 ₃	16 ₂	69	6 ₂	7 ₄
	1901 25	706	13 ₃	25 ₃	246	8 ₂	10 ₄
Females	1911 25	588	0	0	262	1	0
	1901 10	193	5 ₁	11 ₁	64	0	3 ₁
	1901 25	781	5 ₁	11 ₁	326	1	3 ₁
PRESENT SMOKERS ONLY							
Males	1911 25	356	2	8 ₁	123	2	3
	1901 10	130	5 ₃	10 ₂	44	4 ₂	3 ₂
	1901 25	486	7 ₃	18 ₃	167	6 ₂	6 ₂
Females	1911 25	477	0	0	199	1	0
	1901 10	144	5	8 ₁	52	0	3 ₁
	1901 25	621	5	8 ₁	251	1	3 ₁

Tabl 7 14 Mortality¹ from Cancer of the Lung by Sex Age Zygosity and Smoking Discordance Groups; Old Swedish Twin Registry B-series

		DIZYGOTES			MONOZYGOTES		
	Born	No of Pairs	Pooled Low Group	Pooled High Group	No of Pairs	Pooled Low Group	Pooled High Group
PRESENT AND FORMER SMOKERS							
Males	1911 25	501	0	2	177	1	0
	1901 10	20	2	7	69	1	2
	1901 25	706	2	9	246	1	2
Females	1911 25	588	0	0	262	0	0
	1901 10	193	0	1	64	1	0
	1901 25	781	0	1	326	1	0
PRESENT SMOKERS ONLY							
Males	1911 25	356	0	2	123	0	0
	1901 10	130	1	7	44	1	2
	1901 25	486	1	9	167	1	2
Females	1911 25	477	0	0	199	0	0
	1901 10	144	0	1	52	1	0
	1901 25	621	0	1	251	1	0

1) See footnote to tabl 7 11

Table 7 12 Mortality Rates Among Nonsmokers and Mortality Ratios¹ from Selected Causes by Sex Age and Smoking Groups; Old Swedish Twin Register A-series²

		Present Smokers				Former Smokers		
Born		Non-smokers	<10 cpts	>10 cpts	Cigar/pipe	<10 cpts	>10 cpts	Cigar/pipe
		Prevalence Rate	Ratios			Ratios		
<hr/>								
Coronary Heart Disease								
Males	1911-25	1 1	2 1	2 6	1 5	1 5	1 2	1 7
	1901-10	3 7	2 0	2 9	2 5	1 8	3 8	1 5
Females	1911-25	0 3	1 0	1 0	-	1 3	(7 7)	
	1901-10	2 7	2 2	1 0	-	1 4	-	
Cerebrovascular Disease								
Males	1911-25	0 3	0 7	1 7	0 7	1 0	-	
	1901-10	1 2	1 6	1 4	2 1	1 2	0 8	1 3
Females	1911-25	0 3	1 3	5 3	-	1 3	-	
	1901-10	1 7	0 6	0 8	-	-	-	-
Cancer of the Lung								
Males	1911-25	0 06	5 5	12 9	7 7*	-	-	21 7
	1901-10	0 23	6 6	24 8	5 6	2 1	4 4	-
Females	1911-25	0 04	-	7 4	-	-	-	
	1901-10	0 11	6 6	12 6	-	-	-	-
Other Cancers								
Males	1911-25	1 2	0 5	1 3	1 1	1 5	1 8	0 5
	1901-10	3 6	1 2	1 3	1 2	1 8	1 4	0 6
Females	1911-25	1 8	1 3	1 1	-	1 1	(1 3)	-
	1901-10	4 5	0 7	1 8	-	1 8	-	-
Suicides								
Males	1911-25	0 4	2 5	2 8	1 8	0 8	2 0	-
	1901-10	0 7	0 3	1 9	0 9	0 7	1 4	
Females	1911-25	0 2	2 0	8 0	-	-	(11 5)	
	1901-10	0 2	-	-	-	-	-	
Accidents								
Males	1911-25	0 5	1 4	2 6	1 2	0 6	2 6	
	1901-10	1 5	0 4	1 7	0 7	0 6	1 3	
Females	1911-25	0 3	0 7	2 0	-	-	-	
	1901-10	0 5	1 4	-	-	-	(22 2)	-
Other Causes								
Males	1911-25	1 2	1 9	0 9	0 8	1 1	1 8	1 1
	1901-10	5 3	0 8	1 5	0 6	0 5	1 0	0 8
Females	1911-25	0 8	1 5	0 4	-	1 4	-	-
	1901-10	3 5	0 7	2 3	-	-	-	-

1) Not adjusted for age

2) Numbers at risk see table 7 9

Cancer other than that of the lung In the male dizygotic group the B-series analysis reveals a higher number of deaths in the pooled low group (table 7 15). The same tendency is not seen in the female group nor in the monozygotes or in the A-series where the picture is inconsistent. Exclusion of former smokers does not make the findings more clear.

Suicides The A-series analysis (table 7 12 page 98) despite small numbers shows rates that significantly relate to smoking. It is most apparent in the younger age group and is seen for both males and females. Similar results are found in the B-series (table 7 16). For the total cohort of males and females taken together the dizygotes show 11 cases of suicides in the pooled high group as against 4 cases in the pooled low group. Corresponding number for the monozygotes are 6 cases versus 1. Only two cases of suicide occurred among former smokers.

Accidents In the A-series a relation between accidents and smoking is seen mainly among male cigarette smokers both former and present and in both age groups. In the B-series analysis (table 7 17) the numbers are very low but do not as far as the dizygotes are concerned contradict the findings in the A-series. Data from present smokers only does not change the picture.

Table 7 17 Mortality¹ from Accidents by Sex Age Zygosity and Smoking Discordance Groups; CIA Swedish Twin Registry B-series

		DIZYGOTES			MONOZYGOTES		
	Born	No of Pairs	Pooled Low Group	Pooled High Group	No of Pairs	Pooled Low Group	Pooled High Group
PRESENT AND FORMER SMOKERS							
Males	1911-25	501	3	4	177	2	3
	1901-10	203	0	4	69	1	0
	1901-25	704	5	6	246	3	3 ¹
Females	1911-25	588	2	3	262	2	0
	1901-10	193	0	2	64	0	1
	1901-25	781	2	3	326	2	1
PRESENT SMOKERS ONLY							
Male	1911-25	356	4	3	123	1	2
	1901-10	130	0	3	44	1	0
	1901-25	486	4	6	167	2	2
Female	1911-25	477	2	3	199	2	0
	1901-10	144	0	1	52	0	1
	1901-25	621	2	4	251	2	1

1) See footnote to table 7 11

Table 7 15 Mortality¹ from Cancer Other than from the Lung by Sex Age Zygosity and Smoking Discordance Groups; Old Swedish Twin Registry B-series

		DIZYGOTES			MONOZYGOTES		
	Born	No of Pairs	Pooled Low Group	Pooled High Group	No of Pairs	Pooled Low Group	Pooled High Group
PRESENT AND FORMER SMOKERS							
Males	1911-25	501	8	3 ₁	177	0	1
	1901-10	205	10 ₁	6 ₁	69	1 ₁	3
	1901-25	706	18 ₁	9 ₂	246	1 ₁	4
Females	1911-25	588	7 ₃	12 ₁	262	9	8 ₁
	1901-10	193	13 ₁	8 ₁	64	2	3 ₁
	1901-25	781	20 ₄	20 ₁	326	11	11 ₁
PRESENT SMOKERS ONLY							
Males	1911-25	356	5	2	123	0	0
	1901-10	130	6 ₁	0 ₁	44	1	2
	1901-25	486	11 ₁	2 ₁	167	1	2
Females	1911-25	477	6 ₃	8 ₁	199	6	8
	1901-10	144	12 ₁	7 ₁	52	1	1
	1901-25	621	18 ₄	15 ₁	251	7	9

Table 7 16 Mortality¹ from Suicides by Sex Age Zygosity and Smoking Discordance Groups; Old Swedish Twin Registry B-series

		DIZYGOTES			MONOZYGOTES		
Born		No of Pairs	Pooled Low Group	Pooled High Group	No of Pairs	Pooled Low Group	Pooled High Group
PRESENT AND FORMER SMOKERS							
Males	1911-25	501	1	6	177	0	3
	1901-10	205	1	0	69	1	0
	1901-25	706	2	6	246	1	3
Females	1911-25	588	2	5	262	0	3
	1901-10	193	0	0	64	0	0
	1901-25	781	2	5	326	0	3
PRESENT SMOKERS ONLY							
Males	1911-25	356	1	5	123	0	3
	1901-10	130	1	0	44	1	0
	1901-25	486	2	5	167	1	3
Females	1911-25	477	2	5	199	0	2
	1901-10	144	0	0	52	0	0
	1901-25	621	2	5	251	0	2

1) See footnote to table 7 11

Table 7 19 Mortality Ratios in Total and by Smoking in "Factor-positive versus "Factor-negative Individuals and Proportion of "Factor-positive cases in Total Group"; Old Swedish Twin Registry A-series (Condensed from Floderus 1974)

Factor	Mortality Ratios			Proportion of "Factor-positive cases
	Total	Non-smokers	Smokers	
Financially I have not achieved what I hoped for	1.4	1.8	1.3	0.23
My position has involved too much responsibility	1.3	1.6	1.2	0.38
I have had a lot of difficulty in my marriage	1.4	3.5	0.9	0.09
I have often been restless	1.4	1.8	1.3	0.18
I have often had difficulties in falling asleep	1.6	2.0	1.4	0.12
I have often felt somewhat uneasy in my work	1.7	2.3	1.5	0.07

Table 7 20 Age-Standardised Ratios of Observed Over Expected Prevalence Rates Among Nonsmokers Grouped by Smoking Status of Co-twin Gross Mortality Coronary Heart Disease Mortality and Cancer Mortality by Sex and Zygosity; Old Swedish Twin Registry MET-series

		DIZYGOTES		MONOZYGOTES		TOTAL	
		Non-Smoker	All Present Smokers	Non-Smokers	All Present Smoker	Non-Smokers	All Present Smokers
Gross Mortality	Male	0.90	1.11	0.98	1.00	0.92	1.09
	Females	0.92	1.34	0.94	1.30	0.93	1.33
	Total	0.91	1.27	0.95	1.21	0.92	1.27
Coronary Heart Disease	Males	0.78	1.56	0.81	1.51	0.78	1.55
	Females	1.04	1.39	1.00	0.31	1.03	1.10
	Total	0.94	1.45	0.94	0.70	0.94	1.26
Cancer	Males	0.76	0.99	0.63	0.95	0.69	1.01
	Females	0.94	1.89	1.05	0.93	0.98	1.60
	Total	0.90	1.67	0.97	0.94	0.92	1.48

- 1) The total group size is dependent of varying non-response rates for the different items but is mostly ca 5 000 individuals (for details see Floderus 1974)

Age adjusted mortality rates in the A-series fashion by smoking and two different expressions of alcohol behavior namely registration and excessive alcohol consumption (section 4 3 2) appear in table 7 18

Registration appears to be consistently associated with increased mortality in all age and smoking groups except for cigar/pipe smokers in the younger cohort and except for the highest cigarette consumption group in the older cohort. The relationship is also found among nonsmokers. It is also apparent that registration is related to smoking. About one tenth of the nonsmokers are registered as against one fourth in the high cigarette consumption group.

No such obvious trend was seen in regard to excessive alcohol consumption. Only among those who smoked 10 or more cigarettes a day in the youngest cohort was a significant difference observed. No effect of alcohol consumption was seen among nonsmokers.

Table 7 19 presents mortality ratios in the A-series fashion calculated as the mortality rate among individuals who are positive with regard to a certain psycho-social factor (section 4 4) over the rate among individuals who are negative with regard to the same factor. Rates have been

Table 7 18 Age-adjusted Mortality Rates by Age and Smoking Groups among Registered versus "Not Registered" as well as among Excessive Alcohol Consumers versus Remaining Group; Old Swedish Twin Registry Present and Former Smokers Males only A series

Alcohol status	Non-smokers		<10 cigarettes		>10 cigarettes		Cigar/pipe	
	POS	NEG	POS	NEG	POS	NEG	POS	NEG
<u>Registration</u>								
No at Risk								
1911-25	144	1563	291	1342	264	768	188	859
1901-10	91	790	128	621	69	261	88	568
<u>Rates</u>								
1911-25	6.7	4.4	12.2	5.9	15.0	6.9	5.3	5.9
1901-10	25.1	15.3	27.8	18.0	33.3	33.3	27.1	18.8
<u>Excessive consumption</u>								
No at risk								
1911-25	359	1048	707	692	506	348	428	440
1901-10	146	540	235	370	110	137	178	322
<u>Rates</u>								
1911-25	3.4	3.3	5.0	4.1	8.0	4.6	5.1	3.6
1901-10	9.6	10.5	13.1	14.9	20.6	29.2	16.5	13.7

Table 7.19 Mortality Ratios in Total and by Smoking in "Factor-positive versus "Factor-negative Individuals and Proportion of "Factor-positive cases in Total Group¹; Old Swedish Twin Registry A-series (Condensed from Floderus 1974)

Factor	Mortality Ratios			Proportion of "Factor-positive cases
	Total	Non-smokers	Smokers	
Financially I have not achieved what I hoped for	1.4	1.8	1.3	0.23
My position has involved too much responsibility	1.3	1.6	1.2	0.38
I have had a lot of difficulty in my marriage	1.4	3.5	0.9	0.09
I have often been restless	1.4	1.8	1.3	0.18
I have often had difficulties in falling asleep	1.6	2.0	1.4	0.12
I have often felt somewhat weary in my work	1.7	2.3	1.5	0.07

Table 7.20 Age-standardised Ratios of Observed Over Expected Prevalence Rates Among Nonsmokers Grouped by Smoking Status of Co-twin: Gross Mortality, Coronary Heart Disease Mortality and Cancer Mortality by Sex and Zygosity; Old Swedish Twin Registry SUT-series

		DIZYGOTES		MONOZYGOTES		TOTAL	
		Non-Smokers	All Present Smokers	Non-Smokers	All Present Smokers	Non-Smokers	All Present Smokers
Gross Mortality	Males	0.90	1.11	0.98	1.00	0.92	1.09
	Females	0.92	1.34	0.94	1.30	0.93	1.13
	Total	0.91	1.27	0.95	1.21	0.92	1.27
Coronary Heart Disease	Males	0.76	1.56	0.81	1.51	0.78	1.55
	Females	1.04	1.39	1.00	0.31	1.03	1.10
	Total	0.94	1.45	0.94	0.70	0.94	1.26
Cancer	Males	0.76	0.99	0.63	0.95	0.69	1.01
	Females	0.94	1.88	1.05	0.93	0.98	1.60
	Total	0.90	1.67	0.97	0.94	0.92	1.48

1) The total group size is dependent of varying non-response rates for the different items but is mostly ca 5 000 individuals (for details see Floderus 1974)

Age adjusted mortality rates in the A-series fashion by smoking and two different expressions of alcohol behavior namely registration and excessive alcohol consumption (section 4 3 2) appear in table 7 18

Registration appears to be consistently associated with increased mortality in all age and smoking groups except for cigar/pipe smokers in the younger cohort and except for the highest cigarette consumption group in the older cohort. The relationship is also found among nonsmokers. It is also apparent that registration is related to smoking. About one tenth of the nonsmokers are registered as against one fourth in the high cigarette consumption group.

No such obvious trend was seen in regard to excessive alcohol consumption. Only among those who smoked 10 or more cigarettes a day in the youngest cohort was a significant difference observed. No effect of alcohol consumption was seen among nonsmokers.

Table 7 19 presents mortality ratios in the A-series fashion calculated as the mortality rate among individuals who are positive with regard to a certain psycho-social factor (section 4 4) over the rate among individuals who are negative with regard to the same factor. Rates have been

Table 7 18 Age-adjusted Mortality Rates by Age and Smoking Groups among Registered versus Not Registered as well as among Excessive Alcohol Consumers versus Remaining Group; Old Swedish Twin Registry Present and Former Smokers Males only A-series

Alcohol status	Non-smokers		<10 cigarettes		>10 cigarettes		Cigar/pipe	
	POS	NEG	POS	NEG	POS	NEG	POS	NEG
<u>Registration</u>								
No at Risk								
1911-25	144	1563	291	1342	264	768	188	859
1901-10	91	790	128	621	69	261	88	568
<u>Rates</u>								
1911-25	6.7	4.4	12.2	5.9	15.0	6.9	5.3	5.9
1901-10	25.1	15.3	27.8	18.0	33.3	33.3	27.1	18.8
<u>Excessive consumption</u>								
No at risk								
1911-25	359	1048	707	692	506	348	428	440
1901-10	146	540	235	370	110	137	178	322
<u>Rates</u>								
1911-25	3.4	3.3	5.0	4.1	8.0	4.6	5.1	3.6
1901-10	9.6	10.5	13.1	14.8	20.6	29.2	16.5	13.7

Table 7 19 Mortality Ratios in Total and by Smoking in "Factor-positive versus "Factor-negative Individuals and Proportion of "Factor-positive cases in Total Group"; Old Swedish Twin Registry A-series (Condensed from Floderus 1974)

Factor	Mortality Ratios			Proportion of "Factor-positive cases
	Total	Non-smokers	Smokers	
Financially I have not achieved what I hoped for	1.4	1.8	1.3	0.23
My position has involved too much responsibility	1.3	1.6	1.2	0.38
I have had a lot of difficulty in my marriage	1.4	3.5	0.9	0.09
I have often been restless	1.4	1.8	1.3	0.18
I have often had difficulties in falling asleep	1.6	2.0	1.4	0.12
I have often found it somewhat uneasy in my work	1.7	2.3	1.3	0.07

Table 7 20 Age-standardized Ratios of Observed Over Expected Prevalence Rates Among Nonsmokers Grouped by Smoking Status I Co-twin Gross Mortality Coronary Heart Disease Mortality and Cancer Mortality by Sex and Zygosity; Old Swedish Twin Registry KEY-series

		DIKYGOTTES		NEMOYGOTTES		TOTAL	
		Non-Smokers	All Present Smokers	Non-Smokers	All Present Smokers	Non-Smokers	All Present Smokers
Gross Mortality	Males	0.90	1.11	0.98	1.00	0.92	1.09
	Females	0.92	1.34	0.94	1.30	0.93	1.33
	Total	0.91	1.27	0.95	1.21	0.92	1.27
Coronary Heart Disease	Males	0.76	1.56	0.81	1.51	0.78	1.55
	Females	1.04	1.39	1.00	0.31	1.03	1.10
	Total	0.94	1.48	0.94	0.70	0.94	1.26
Cancer	Males	0.76	0.99	0.63	0.95	0.69	1.01
	Females	0.94	1.08	1.05	0.93	0.98	1.00
	Total	0.90	1.67	0.97	0.94	0.92	1.48

1) The total group size is dependent of varying non-responses rates for the different items but is mostly ca 5 000 individuals (for details see Floderus 1974)

7 3 4 Mortality and some risk factors other than smoking

Age adjusted mortality rates in the A-series fashion by smoking and two different expressions of alcohol behavior namely registration and excessive alcohol consumption (section 4 3 2) appear in table 7 18

Registration appears to be consistently associated with increased mortality in all age and smoking groups except for cigar/pipe smokers in the younger cohort and except for the highest cigarette consumption group in the older cohort The relationship is also found among nonsmokers It is also apparent that registration is related to smoking About one tenth of the nonsmokers are registered as against one fourth in the high cigarette consumption group

No such obvious trend was seen in regard to excessive alcohol consumption Only among those who smoked 10 or more cigarettes a day in the youngest cohort was a significant difference observed No effect of alcohol consumption was seen among nonsmokers

Table 7 19 presents mortality ratios in the A-series fashion calculated as the mortality rate among individuals who are positive with regard to a certain psycho-social factor (section 4 4) over the rate among individuals who are negative with regard to the same factor Rates have been

Table 7 18 Age-adjusted Mortality Rates by Age and Smoking Groups among Registered versus Not Registered as well as among "Excessive Alcohol Consumers" versus "Remaining Group"; Old Swedish Twin Registry Present and Former Smokers Males only A series

Alcohol status	Non-smokers		<10 cigarettes		>10 cigarettes		Cigar/pipe	
	POS	NEG	POS	NEG	POS	NEG	POS	NEG
<u>Registration</u>								
No. at Risk								
1911-25	144	1563	291	1342	264	768	188	859
1901-10	91	790	128	621	69	261	88	568
Rates								
1911-25	6.7	4.4	12.2	5.9	15.0	6.9	5.3	5.9
1901-10	25.1	15.3	27.8*	18.0	33.3	33.3	27.1	18.8
<u>Excessive consumption</u>								
No. at risk								
1911-25	359	1048	707	692	506	348	428	440
1901-10	146	540	235	370	110	137	178	322
Rates								
1911-25	3.4	3.3	5.0	4.1	8.0	4.6	5.1	3.6
1901-10	9.6	10.5	13.1	14.9	20.6	29.2	16.5	13.7

8.1 Introduction

The presentation of the twin research program in this report has so far involved an extensive description of methodological aspects of the twin method as well as a detailed account of various results from a series of questionnaire studies, clinical examinations and mortality follow-ups.

Upon the initiation of the program in 1959 the intention was primarily to perform a follow-up in longitudinal manner of cohort of twins and to study mortality against the background of smoking habits. When the older cohort of twins born 1886-1925 was approached for the first time information was thus sought mainly about smoking habits and some similarity variables for determining zygosity. At various stages during the course of time a series of questionnaires were mailed to the twins asking for information about habitual factors other than smoking as well as about broader spectrum of disease experience. In addition some detailed clinical examinations were performed on subsamples of twins furnishing information to elucidate certain topic areas such as the relationship between tobacco smoking and coronary heart disease, genetic aspects of respiratory and cardiovascular disease, the influence of drinking on the endpoints studied and the importance of psycho-social factors for the smoking-disease association.

The extension of the twin program in 1971 to include cohorts born 1926-1958 was motivated by the need to have base-line information on socio-economic behavior and psychological, sociological and medical factors available for longitudinal investigations in the field of environmental health. Even though this extended program does not primarily focus on smoking and health, in the long run it nonetheless furnishes mortality data that will supplement the analyses performed on the older cohorts of the registry. It has already been used to give information on the question of differences between various smoking and nonsmoking groups in regard to an extended series of variables.

Upon the initiation of the twin program two major assumptions were made. The first was that smokers differ from nonsmokers in many aspects relevant for developing disease and for increasing the risk of premature death. The second assumption was that many such variables would be more similar between the two members of a twin-pair as compared to unrelated individuals even if the twins in a pair were dissimilar in regard to smoking.

The present chapter will first assess to what extent the above mentioned assumptions have been supported by the data. A second part of the chapter will evaluate whether the results from the analysis on twin pairs have given information on the smoking-health relationship that deviates from results obtained in conventional epidemiological analysis. If so the question then is to what extent studies on twin pairs yield information of recognizable epidemiological value and contribute to better understanding of the relationship between smoking and health.

calculated both in total and separately for smokers and nonsmokers. The last column presents the relative number of factor positives in the total group. The table shows the 6 items out of a total of 15 where any of the mortality ratios proved to be statistically significant. It is apparent from the table that psycho-social factors are related to mortality in smokers as well as nonsmokers. The factors lastly are not too uncommon in the population. The above presented analyses were both of the A-series type. Corresponding B-series analysis can hardly be performed at present since the requirement of controlling for both smoking and the "factor" would reduce the numbers drastically.

7 3 5 *Mortality among nonsmoking partners of smoking co-twins*

Table 7 20 page 103 shows the results of a MET-analysis in regard to gross mortality, mortality in coronary heart disease and cancer all sites. It has due to low numbers not been possible to carry out a MET analysis on other specific causes of death.

In the table data are given for the nonsmoking partners of nonsmoking co-twins as well as for the nonsmoking partners of presently smoking co-twins. The groups are broken down by sex and zygosity and are also presented in subtotals and totals. As usual standardization is performed over sex and age groups. It is obvious from several subgroups that the mortality among the nonsmokers is significantly related to the smoking status of the partner. Significant ratios are not seen among the monosygotes. It should however be pointed out that some ratios are increased also in the monosygotes.

common among the high smokers and extravertness appeared to be moderately but significantly more common in the smoking groups as compared to nonsmokers.

This consistent picture was shown for men as well as for women. There were no striking contrasts between age groups except that higher ratios were often found in the very youngest age groups comprising boys and girls in the age range of 15-17 years. For example, in this age bracket drinking was up to 5 times more common among smokers than among nonsmokers (77 percent as against 13 percent); use of oral contraceptives was up to 9 times more common among the female smokers than among the female nonsmokers (16 percent as against less than 2 percent).

8.2.3 Summary of results from studies on twin pairs

B-analyses of smoking discordant twin pairs, the purpose of which is to measure the degree of control for the variables studied, were performed on nonsmokers against present cigarette smokers. Observed ratios of the prevalence of risk factors among the smokers over the prevalence of such among the nonsmokers, adjusted for sex, age and smoking quantity, were compared to expected ratios based on A-series rates.

The results showed clearly that the observed ratios for most items were considerably lower than the expected ones and more often lower among monozygotes as compared to dizygotes. For example, in regard to drinking the monozygotic ratio was 1.1 as compared to an expected ratio of 1.5. For excessive drinking the ratio was 1.3 as against 4.5. Examples of less good control are sleeping pills and tranquilizers with observed ratios of 1.3 and 1.8 as against the expectations of 2.3 and 1.9.

For traits where the ratio of the prevalence of smokers over that of nonsmokers in smoking discordant pairs was less than its expectation, the prevalence among the nonsmoking partners was often higher than that among all nonsmokers. This was studied for three groups of discordant pairs: the partner being either a present cigarette smoker, present smoker regardless of type, or a former smoker.

It appeared, for example, that excessive drinking was 2.6 times more prevalent among monozygotic nonsmokers with partners presently smoking cigarettes as compared to all monozygotic nonsmokers in the twin series. A further example from the same subgroup is provided by sleeping pills with a ratio of 1.2. Generally, the monozygotic ratios tended to be higher than the dizygotic ones. Only in exceptional cases were the ratios for nonsmoking partners of former smokers significantly increased.

For both monozygotes and dizygotes, the coincidence ratios with very few exceptions were higher than expected and almost consistently higher in the monozygotic series.

The clinical examinations compared smokers and nonsmokers with regard to, for example, blood pressure, serum lipids and uric acid, which are known biological risk factors in coronary heart disease. Both blood pressure and cholesterol level were, if anything, negatively correlated to smoking in monozygotic pairs, while phospholipids were moderately increased among smokers. Not one single biological finding showed such pronounced difference between the smokers and nonsmokers as did the socioeconomic, habitual and psycho-social items presented above.

8 2 Group Comparability between Various Smoking and Nonsmoking Groups

8 2 1 Introductory remarks

In the introduction of chapter 6 a short review was given of the literature in regard to differences between smokers and nonsmokers. Substantial evidence showing that smokers differ from nonsmokers in respect to many variables was found to exist.

In the present report the group comparability has been further studied on a large number of variables and with due consideration paid to possible relations to amount and type of smoking as well as to associations with sex and age. Thus the present study evaluates whether differences found between unrelated smokers and nonsmokers (A series) still existed or were fully or partly controlled between the partners in smoking discordant monozygotic and dizygotic twin pairs (B-series). A possible reflection of such control could also be that the nonsmoker in a smoking discordant pair due to genetic or environmental factors shows similarities with his smoking partner while he at the same time differs from all nonsmokers of which the majority belongs to concordantly nonsmoking pairs (NET-analysis).

8 2 2 Summary of results from studies on unrelated individuals

The question of differences between smokers and nonsmokers has been elucidated on the basis of data mainly from the new twin registry. The investigation covered several subject areas such as socioeconomic data and variables connected with education and occupation, data on consumption of alcohol and some drugs, certain food habits and variables indicating psycho-social discord.

The socioeconomic picture of the smokers shows them more often to be living in apartments than in private homes, less often to have an education beyond elementary school, more often to change employment and to have overtime, shiftwork and piecework.

The most dramatic association was shown in regard to drinking and especially excessive drinking. Consumers of more than 16 cigarettes a day drank excessive amounts of alcohol 3-10 times more often than the nonsmokers. Sleeping pills and tranquillizers were used 3.5 times as often among the high cigarette smokers as among the nonsmokers. The use of oral contraceptives among women was strongly related to amount of smoking.

In regard to food, smokers displayed higher prevalence of such somewhat "unhealthy" habits as having only one warm meal or less a day and drinking five or more cups of coffee a day. Furthermore they displayed lower use of "healthy" food items such as fruits and vegetables.

Indications of psycho-social discord were also related to smoking. Sleeping difficulties and stress were thus reported about twice as often by the smokers in the highest cigarette smoking category as compared to nonsmokers. Divorce was about four times as common among high smokers as among nonsmokers. A personality measurement according to a special psycho-social scale showed that instability was up to three times as

8 3 Smoking Related Morbidity and Mortality

8 3 1 Introductory remarks

The results discussed in foregoing sections have clearly indicated that both assumptions presented in section 8 1 were strongly supported

Thus the first assumption was borne out because smokers and nonsmokers did differ in regard to almost all variables measured in the 1973 questionnaire study and the question is to what extent such variables may have an influence on the health status of the individual. Some of them have previously been found to be related to disease and premature death socioeconomic and occupational variables (Larsson 1965; US Dept of H&M 1963) habitual factor (Klatsky Friedman and Siegelman 1973 1974) and psycho-social status (Friedman and Rosenman 1959; Jenkins 1971; Otfield et al 1964; Jenkins Rosenman and Tytanski 1968)

The second assumption that the modified twin control method as defined in the present report should offer a better group comparability than the conventional comparison of study groups - was also verified even if the control was not complete

The genotype identity among monozygotes by definition leads to an ideal group comparability in regard to constitutional factors. As for the habitual and psycho-social items studied almost all showed coincidence values that were significantly above expectation and almost consistently higher among monozygotes as compared to dizygotes. The degree of control as discussed in section 8 2 3 was often pronounced particularly in monozygotic pairs

The following sections will summarize results from the morbidity and mortality studies performed on the cohort born 1901-1925. The cohort born 1896-1900 was not included in the analysis due to their high age at the induction of the study and due to the fact that the numbers were low and would with few exceptions not have contributed much to the analysis

Section 8 3 2 will present results in regard to morbidity and mortality which are similar to what would have been obtained in a study on unselected nonsmoking and smoking individuals. Section 8 3 3 will deal with the analyses on smoking discordant twin pairs in regard to gross mortality and more specifically in regard to mortality from lung cancer and coronary heart disease. The conclusions will be presented in section 8 3 4

8 3 2 Summary of results from studies on unrelated individuals

The morbidity investigations show a direct relationship between smoking and the symptoms cough and angina pectoris in both the Swedish registries and in the American registry. Furthermore in the new Swedish registry stomach disorder back disorders impaired hearing migraine asthma long-lasting illness and sick leave more than or equal to 3 months in row are all smoking related. It is worth emphasizing that hypermorbidity ratios around 2 and 3 are not infrequently found among the last mentioned diseases and that some high ratios are also found in the very youngest age group and among individuals who smoke 7 cigarette day or less. Such an example is back disorders which appear with a

The results of the comparison of smokers and nonsmokers as unrelated individuals show beyond doubt that smokers are different from nonsmokers within rather broad conceptual areas. Men and women bear out this finding to about the same extent; very young smokers do not deviate from this picture but rather strengthen it. There is no reasonable way of attributing these findings to methodological fallacies nor is there any reason to question their generalizability to the population at large. The explanation of the findings can theoretically be environmental or genetic or both. The smokers may simply have been living in a different environment from the nonsmokers but genetic differences may also have played a large role.

There is no unobjectionable way of discriminating between the environmental and genetic influences in the twin model as applied here. Certainly the coincidence in regard to several of the above mentioned traits especially the behavioral and psycho-social ones was significantly higher among the monozygotic pairs as compared to the dizygotic. According to the classical twin method such a finding would indicate an influence of genetic factors. However genetic heredity may be less influential than a construct variable that could be termed social heredity. On the other hand it may be argued that social heredity may itself reflect genetic determination to some extent. As the coincidence of the mentioned traits were also above expectation among the dizygotic twins this finding can hardly be explained solely by the genetic resemblance between siblings. The concept of social heredity may thus reasonably be considered as supported. The excess coincidence among the monozygotic pairs as compared to the dizygotic may also be environmentally influenced. A certain pressure may have been exerted not only from within the pairs but also from an environment that expected the twins to behave alike.

In regard to several traits the prevalence rate among nonsmokers in the discordant group proved to be significantly increased in comparison to all nonsmokers in the twin series. These findings - whether genetically or environmentally determined - motivate an assumption of a life-style parameter that expresses itself in the development of not only smoking behavior but also of a great variety of habitual traits. Smoking could then be regarded as one of many indices of this parameter.

and 26/22 respectively. Analyses of significance show that the excess mortality is significant only for dizygotes and not for monozygotes but also that a direct statistical comparison between the distributions for the monozygotic and the dizygotic group does not reveal significant results. In the group of smokers versus nonsmokers the dizygotes show a hypermortality of 55/37 for males and 52/43 for females. Corresponding numbers among monozygotic pairs were 15/10 and 22/18 respectively. The difference is significant in male dizygotes only. As for the observed hypermortality among exposed monozygotes it is worth pointing out that the excess was found mainly in the younger age-group.

The increased mortality in the monozygotic group was hardly seen during the first ten years of observation. In the last five-year period however the hypermortality calculated as incidence ratios was no longer different between the zygosity groups. This is true irrespective of whether former smokers were included or not in the analysis.

The hypermortality in the dizygotic group expressed as a ratio is about 1.5 which is close to what can be expected on the basis both of the A-series and of the Swedish smoking study on 55 000 males and females (Cederlöf et al 1975) allowing mean discordance of about 7.8 cigarettes/day.

Among the cause-specific deaths in the B-series analysis lung cancer reflects the findings in the A-series. Among dizygotic males 9 cases occurred in the pooled high group against 2 cases in the pooled low group or if only smoker versus nonsmokers are considered 6 cases versus 1 case. Among monozygotic males 3 cases occurred: 2 in the pooled high group and 1 in the pooled low group. The case in the low group was also a smoker. However, in view of the importance of lung cancer as smoking-related cause of death, the low number of cases among the monozygotes prompted the analysis of data from the oldest cohort of twins born 1886-1900 (Cederlöf and Friberg to be published). It was found that 3 lung cancer deaths had occurred among 69 pairs in the dizygotic group and 3 deaths among 50 pairs in the monozygotic group. All cases occurred in the high exposure group. Taking 11 age groups together there were 12 cases in the pooled high group against 2 cases in the pooled low group among dizygotes. Corresponding numbers for the monozygotes were 5 against 1. Among females only 2 cases appeared: 1 in the dizygotic pooled high group and 1 in the monozygotic pooled low group, the last mentioned case also being a smoker.

Other diagnoses of respiratory disorders as cause of death have not given great enough numbers to warrant any statistical treatment. It should be mentioned in this connection that the morbidity studies in Sweden as well as in the US showed clearcut relationships not only in the A-series analysis but also in the B-series in regard to cough and prolonged cough. Moreover, the clinical investigation by Lundman (1966) showed that lung function tests were significantly impaired among smokers in both monozygotic and dizygotic pairs.

The mortality data on coronary heart disease analyzed in the B-series fashion on the pooled high and pooled low groups reveal distribution of deaths that is 25/13 for male and 11 for female dizygotic twins. Among monozygotic twins the distribution is 10/8 for males. Among female monozygotes only 4 cases were observed of which 3 belonged to the pooled high smoking group and 1 to the lower group. If only present smoker are considered the numbers in the pooled high and pooled low male groups were 18/7 among dizygotes and 6/6 among monozygotes.

ratio of 2.9 among young males and 2.5 among young females. Stomach disorders in the youngest and lowest smoking female groups show a hypermortality ratio of 1.6. Sick leave for 3 months in a row or more appears with hypermorbidity ratios of 3.3 and 2.5 among the youngest males and females respectively in the lowest cigarette smoking group.

The gross mortality is strongly related to present smoking: the group smoking more than 10 cigarettes a day displaying hypermortality ratios of about 2. This is true for both men and women and in both age-groups. Male former smokers who used to smoke more than 10 cigarettes daily also show similarly increased ratios.

Gross mortality was also related to other risk factors. Thus alcohol registration per se was associated with mortality in nonsmokers as well as smokers. The mortality in several subgroups was about twice as high among registered as compared to non-registered. These data fit well with results from the Swedish smoking study on 55 000 males and females (Cederlöf et al. 1975).

Also certain psycho-social factors such as financial problems, too much responsibility, restlessness and sleeping difficulties were clearly related to mortality.

Cause specific mortality ratios are found to be significantly increased for coronary heart disease, lung cancer, suicides and accidents in several subgroups.

Death from coronary heart disease already shows an increase with relative risks of about 2 among those who smoke or smoked 10 cigarettes or less a day. The ratio approaches 3 among the males who were smoking more than 10 cigarettes daily in 1961. Former smokers born 1901-1910 show similarly increased ratios.

Lung cancer is closely related to amount smoked, showing ratios of 5-6 in the lower and of 13-25 in the higher smoking category among males. Former smokers show considerably lower hypermortality ratios, but the low numbers make a quantification difficult.

Increased ratios were also found for suicides and accidents: 2-8 and about 2 respectively.

8.3.3 Summary of results from studies on twin pairs

The analyses are generally based on separate statistical treatment of two sets of discordance groups, of which the first set is confined to smokers versus nonsmokers; the second set of discordance groups also includes pairs where both are smokers but smoke different amounts (pooled high group versus pooled low group, cf. section 4.3.2). This step has been taken in order to gain in numbers. Data were also treated with and without former smokers included. Since the discrepancies between these groups in most cases were minor and in consideration of the fact that also former smokers in the A series showed elevated mortality ratios, the following comments relate mostly to the group with former smokers included.

Among diszygotic pairs there was an obvious hypermortality in the pooled high group compared with the pooled low group: 82/54 for males and 60/44 for females. Corresponding numbers among monozygotic pairs were 27/24.

and 26 22 respectively. Analyses of significance show that the excess mortality is significant only for dizygotes and not for monozygotes but also that direct statistical comparison between the distributions for the monozygotic and the dizygotic group does not reveal significant results. In the group of smokers versus nonsmokers the dizygotes show a hypermortality of 55 37 for males and 52 43 for females. Corresponding numbers among monozygotic pairs were 15 10 and 22 18 respectively. The difference is significant in male dizygotes only. As for the observed hypermortality among exposed monozygotes it is worth pointing out that the excess was found mainly in the younger age-group.

The increased mortality in the monozygotic group was hardly seen during the first ten years of observation. In the last five-year period however the hypermortality calculated as incidence ratios was no longer different between the zygosity groups. This is true irrespective of whether former smokers were included or not in the analysis.

The hypermortality in the dizygotic group expressed as a ratio is about 1.5 which is close to what can be expected on the basis both of the A-series and of the Swedish smoking study on 55 000 males and females (Cederlöf et al 1975) allowing mean discordance of about 7.8 cigarettes/day.

Among the cause-specific deaths in the B-series analysis lung cancer reflects the findings in the A-series. Among dizygotic males 9 cases occurred in the pooled high group against 2 cases in the pooled low group or if only smokers versus nonsmokers are considered 6 cases versus 1 case. Among monozygotic males 3 cases occurred 2 in the pooled high group and 1 in the pooled low group. The case in the low group was also smoker however in view of the importance of lung cancer smoking related cause of death the low number of cases among the monozygotes prompted the analysis of data from the oldest cohort of twins born 1886-1900 (Cederlöf and Friberg to be published). It was found that 3 lung cancer deaths had occurred among 89 pairs in the dizygotic group and 3 deaths among 50 pairs in the monozygotic group. All cases occurred in the high exposure group. Taking all age groups together there were 12 cases in the pooled high group against 2 cases in the pooled low group among dizygotes. Corresponding numbers for the monozygotes were 5 against 1. Among females only 2 cases appeared 1 in the dizygotic pooled high group and 1 in the monozygotic pooled low group the last mentioned case also being smoker.

Other diagnoses of respiratory disorders as cause of death have not given great enough numbers to warrant any statistical treatment. It should be mentioned in this connection that the morbidity studies in Sweden as well as in the US showed clearcut relationships not only in the A-series analysis but also in the B-series in regard to cough and prolonged cough. Moreover the clinical investigation by Lundman (1964) showed that lung function tests were significantly impaired among smokers in both monozygotic and dizygotic pairs.

The mortality data on coronary heart disease analyzed in the B-series fashion on the pooled high and pooled low groups reveal distribution of deaths that is 25 13 for male and 11 5 for female dizygotic twins. Among monozygotic twins the distribution is 10 8 for male. Among female monozygotes only 4 cases were observed of which 3 belonged to the pooled high smoking group and 1 to the lower group. If only present smokers are considered the numbers in the pooled high and pooled low male groups were 18 7 among dizygotes and 6 6 among monozygotes.

Coronary heart disease as a morbidity entity was investigated in two separate clinical examinations. In the Lundman study (1966) on smoking discordant twin pairs no higher prevalence rates were revealed among the smokers than among the nonsmokers. In another clinical study (Liljefors 1970) on pairs discordant for coronary heart disease no significantly greater numbers of smokers were found among the diseased partners as compared to the nondiseased partners. The Liljefors study instead suggested that variables of psycho-social character such as ambition and dedication to work have a greater impact on the development of the disease.

NET-analyses were performed on morbidity data in the new twin registry in the same fashion as was done for non-medical data. It was shown that the symptoms cough, shortness of breath, stomach disorders, back disorders and migraine as well as sick leave for 3 months or more in a row occurred more often among monozygotic nonsmokers with a presently smoking partner than among all the nonsmokers in the twin series. This was especially marked for males. The ratios were significantly increased with values ranging from 1.3-1.9. Only in regard to stomach disorders were the ratios reliably increased for dizygotes. Significantly increased ratios for back disorders and longlasting illness were found also among nonsmoking partners of former smokers.

A NET analysis was also carried out on gross mortality, mortality in coronary heart disease and cancer all sites. The data have been treated in various subgroupings. Differences were often found to the effect that nonsmokers in smoking discordant pairs had higher mortality rates than had the total group of nonsmokers.

8.3.4 Discussion and conclusions

The analyses of data from the old twin registry when the twins are regarded as a series of unrelated individuals show results that by and large confirm the well established picture of smoking as an indisputable risk factor for disease and premature death. To the extent gross and cause specific mortality data have been studied they correspond largely with results found in the Swedish study on 55 000 Swedish men and women and except for the increased suicidal ratios do not deviate to any greater extent from the results of the well known US and UK epidemiological studies on smokers and nonsmokers. The prevalence rates are almost consistently dose-related. Men and women differ in some respects such as cause-specific deaths from coronary heart disease and cancers other than that of the lung but broadly speaking there is nothing to indicate that women would be considerably less at risk than men within the same smoking categories.

In the new twin registry only morbidity has been possible to study. The associations between smoking and respiratory and cardiovascular symptoms were confirmed. Associations were however also found with a large number of indications of other symptoms of disease or ill health such as stomach disorders, back disorders, impaired hearing and longlasting illness. Such associations were found also in the very youngest age groups, 15-17 years where taking exposure time and the low amounts of smoking into account the exposure must be considered low. In regard to respiratory symptoms it is not unreasonable that even a short exposure could give rise to symptoms in susceptible individuals. It is much more difficult to see how there could be a causal association between smoking and for example back disorders, longlasting illness and sick leave for three or

more months in a row in young people exposed to low amounts of tobacco for just few years. On the contrary these findings support the evidence from the foregoing sections that the smokers should be overrepresented by people who are constitutionally different from nonsmokers or who quite generally indulge in unhealthy living. Theoretically the results may also reflect to some extent a tendency for smokers to overreport physical ill feelings possibly due to differences in personality.

The conventional picture revealed by the A-series analysis is by and large reflected in the B-series analyses of disygotic twins where respiratory morbidity, total mortality and death from several specific causes including lung cancer and coronary heart diseases show increased relative risks among the more exposed partners in the discordant pairs. Generally these relative risks do not differ to any appreciable extent from what can be expected considering a mean discordance of about 7 cigarettes a day.

The monozygotic picture agrees with the disygotic in several respects but not in all. The most striking similarity between the two zygosity groups was found in regard to respiratory symptoms and lung cancer. The well documented evidence of a causal association between smoking and lung cancer found in other studies has been further supported.

In regard to coronary heart disease the situation is less clearcut. Among males the disygotic ratio of 1.9:1 is significant while the monozygotic ratio of 1.2:1 is rather close to zero. If former smokers are excluded the corresponding ratios are 2.6 and 1.0 respectively, the latter ratio however being based on the distribution of 12 cases only. The outcome nevertheless suggests a difference between the two zygosity series even if the monozygotic numbers are too small to warrant more firm conclusions.

Concerning the gross mortality it has earlier been pointed out (Friberg et al 1973) that no hypermortality at that time was seen in the monozygotic pooled high group. There is still no statistically significant hypermortality related to smoking in the monozygotic groups. Looking at the picture as a whole however (table 7.11) there are three consistent tendencies towards excess death also in the monozygotic group. This is due to an increased incidence during the last 5-year period.

It is obvious that suicides considerably and to some extent also accidents contribute to the hypermortality in smokers. Pooling males and females and monozygotes and disygotes 17 suicides have taken place in the pooled high groups as against 5 in the pooled low groups which gives a ratio of 3.4. Although suicides and accidents are associated with smoking it has not been considered reasonable to assume that they are causally related to smoking. Certainly from the theoretical point of view suicides can be assumed to be precipitated by the awareness of serious somatic disease and that smoking may cause accidental fires. There was nothing whatsoever in the medical records including information from autopsies that indicated such an explanation. Instead it indicates that among smokers there is an overrepresentation of individuals with an increased risk for committing suicides. The findings seem to be yet another indication of the incomparability of smokers and nonsmokers.

Still another problem in comparing smokers and nonsmokers is reflected by the high rate of "registered" among the smokers. In the twin studies and the Swedish probability sample (Cederlöf et al 1975) it is shown beyond doubt that registration with all its implications of alcohol

abuse and asocial behavior is not only associated with smoking but is a considerable risk factor per se as demonstrated by increased rates also among registered nonsmokers. Because of low numbers it has not as yet been possible to make a detailed analysis in the B-series fashion.

There are some unpublished mortality data from the US twin registry which are of interest also in this context (Hrubec, Cederlöf and Friberg to be published). In the analysis of 1403 dizygotic and 1113 monozygotic smoking discordant twin pairs 61 deaths were observed in the dizygotic pooled high group as against 42 in the pooled low group. Corresponding numbers in the monozygotic group were 45 against 31. Thus a hypermortality related to smoking was found in both zygosity groups. When however pairs with discordant or unknown drinking habits were excluded from the total group it was found that 25 deaths had occurred in the high smoking group as against 25 deaths in the low smoking groups among 729 smoking discordant dizygotic twin pairs. Corresponding numbers of deaths among 640 monozygotic pairs were 21 and 20 respectively. If the group "high smokers and high drinkers" are compared to their low smoking and low drinking co-twins the numbers of deaths were 21 as against 10 for dizygotes and 11 as against 5 for monozygotes. The reason for the different results in the different subgroups is not clear. It is however not possible to explain it by differences in smoking habits. At least two possible explanations exist, namely that there is either an interactive effect of alcohol and tobacco or selective effects implying that the presence of both habits in an individual indicates a certain constitutional selection.

A question of importance for the total assessment of the differences found between the monozygotic and dizygotic groups is to what extent the smoking discordance is comparable. This has been discussed extensively in section 4.6.2 and it can be concluded that even if minor differences may have existed, they should not have been of decisive importance. In addition the data on lung cancer further support such a statement.

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- 1) The discordance classification differs somewhat from the one used in the present report.

8.4 Smoking and Health in the Perspective of Twin Research

Studies on smoking and health have to a great extent focused on lung cancer and coronary heart disease. Gross mortality obviously is also of great concern not only due to the fact that the two mentioned diseases particularly coronary heart disease constitute major causes of death but also due to the fact that a great variety of other diseases have been claimed to be causally associated with smoking. In the present evaluation it has however not been possible to divide gross mortality into more than a few subgroups.

As for lung cancer, so much data have accumulated during the last decades that there should be no doubt about a causal link between smoking and the development of the disease. However, the constitutional hypothesis, as advanced by Fisher and still supported by a few, has here been tested in twin studies. The results from the Swedish monozygotic twin series speak strongly against this constitutional hypothesis. Constitution may still be of importance, however, in the way that certain individuals may be more susceptible. It has thus been pointed out that the enzyme aryl hydrocarbon hydroxylase (AHH) may be genetically determined and mediate an increased susceptibility to certain carcinogens found in tobacco smoke.

Chronic bronchitis is generally considered to be closely associated with smoking. This disease entity is not a common cause of death in Sweden and no evaluation can be made based on the present mortality data. The prevalence of different degrees of cough was however strongly associated with smoking in both dizygotic and monozygotic smoking discordant pairs. There could be no doubt that such respiratory symptoms are causally associated with smoking, even if the data suggest that constitutional factors for a susceptible individual may be of the same importance as is smoking for the development of the disease.

Mortality in coronary heart disease has in a number of epidemiological studies been associated with smoking. A similar association was found among unrelated individuals in the Swedish twin studies as well as in the recent Swedish study on 3 000 men and women. Even though the hypermortality rates are generally considerably lower than those for lung cancer, nonetheless the absolute number of excess deaths among smokers is considerably higher on the population scale due to the high incidence of coronary heart disease.

The role of smoking in the pathogenesis of this disease is such more open as it is a multifactorial disease where several risk factors play an important part and where these may be strongly interrelated. One of the reasons for initiating the present research program was the assumption that such risk factors to a great extent could be correlated with smoking and that when studying smoking discordant pairs the risk factors would be more similar between the members of smoking discordant monozygotic pairs than in dizygotic pairs or between smokers and nonsmokers among unrelated individuals. The results from smoking discordant twins suggest that the hypermortality among monozygotic pairs may distribute differently as compared with the dizygotic pairs and the A-series. These data are however not yet conclusive due to low numbers. Great care should be exercised in interpreting this finding, especially as the gross mortality incidence ratio has increased in the monozygotic group during the

last five years of observation indicating possible biasing effects due to censoring. Such bias is more likely to influence the outcome during the first years of observation. As in the dizygotic group there was a hypermortality already during the first period. The outcome may just as well indicate that other factors than smoking play an essential part in the etiology of coronary heart disease i.e. factors which are less discordant in the monozygotic than in the dizygotic pairs. Such an assumption is also congruent with the results reported by Lundman (1966) and Liljeferg (1970) in their clinical studies on smoking discordant and coronary heart disease discordant twins. There is also all reason to believe that genetic factors may play an important role also in the development of coronary heart disease. This was well documented by de Faire (1974) in his study on death discordant twins where he found that the surviving partner of deceased twins with a diagnosis of coronary heart disease more often had symptoms and signs of this disease.

Suicides and to some extent accidents occur to a significantly higher extent among smokers. Out of 7 suicides in the monozygotic discordance groups 5 are found among the high exposed twins. If smoking is held to be unlikely as a causative factor for suicides, the estimate of hypermortality is no doubt unduly influenced by the suicide rate.

As the major part of the data from the Swedish twin registry is based on questionnaire information and subsequent mortality follow-ups, it has not been possible to study to any great extent physiological risk factors. Instead the emphasis has been put on behavioral and psycho-social variables as well as on genetic interactions.

The results clearly show that differences have been revealed for a large number of behavioral and psycho-social items also in very young people smoking only a few cigarettes a day. The smokers deviated from the nonsmokers not only with regard to behavioral and psycho-social data; they did so also with respect to some effect variables such as back disorders and continuous sick leave for long periods where the causal link to smoking seems remote. Further support for differences between smokers and nonsmokers was provided by very high hypermortality ratios for suicides. The associations between smoking and registration in an alcohol registry point in the same direction. The finding that several habitual risk factors as well as morbidity and mortality in the RET analysis were increased among nonsmokers if they had partners that were classified as present smokers indicates that smoking may be influenced by both genetic and social heredity.

The results fit a model in which nonsmokers and smokers are different in regard to a life-style parameter of which smoking is but one index others being alcohol drinking, drug abuse, psycho-social discord etc. It may be assumed that this parameter ranges from a healthy value at the one extreme to an "unhealthy" value at the other extreme. The concordant nonsmokers probably have values close to the first mentioned extreme and concordant smokers close to the other, leaving the discordant smokers to occupy more undecided values in the middle of the range. If this construct variable is associated with disease which is highly probable, it is obvious that nonsmokers in discordant pairs should experience ill health to a higher degree than concordant non smokers. Whether the basic explanation is predominantly genetic or environmental may be open for speculation. However, from the analysis of the twin pairs it does seem that monozygotes as a rule fit the model better than dizygotes as demonstrated by better control of most habitual

and psycho-social traits high coincidence and more pronounced life-style effects in the MZ-analysis. These data point towards at least some influence of genetic factors.

Even if quantitative assessments presently cannot be made of the relative roles of smoking, other risk factors and genetic disposition, it seems that epidemiological investigations that have not controlled for factors referred to above may have overestimated the role of smoking as a causative factor in coronary heart disease and also considering for example the distribution of suicides among smokers and nonsmokers that such studies have more than duly made smoking responsible as a causative factor for all excess deaths seen in smokers. On the other hand this should not be taken as evidence that smoking should be irrelevant as precipitating factor for deaths in for example coronary heart disease. It seems for example obvious that carbon monoxide level reached in blood already in moderate smokers may be deleterious in subjects with impaired coronary circulation (WHO 1975).

The results from the twin study clearly demonstrate the importance of genetic, several behavioral and psycho-social factors which have not been considered in conventional epidemiological studies. Such factors should as far as possible be included in future epidemiological research not only in the context of smoking and health but also in studies on other similar exposure factors that may be linked to risk factors of this type or to genetic predispositions. The twin approach certainly enhances the group comparability to considerable extent not only in regard to genetic but also with respect to habitual and psycho-social factors that may be of etiologic relevance. The major drawback of the twin method is the question of numbers. A difficulty however that can be solved by integrating data from several research centers using the twin method. Such programs are under way.

last five years of observation indicating possible biasing effects due to censoring. Such bias is more likely to influence the outcome during the first years of observation. As in the dizygotic group there was a hypermortality already during the first period. The outcome may just as well indicate that other factors than smoking play an essential part in the etiology of coronary heart disease i.e. factors which are less discordant in the monozygotic than in the dizygotic pairs. Such an assumption is also congruent with the results reported by Lundman (1966) and Liljefors (1970) in their clinical studies on smoking discordant and coronary heart disease discordant twins. There is also all reason to believe that genetic factors may play an important role also in the development of coronary heart disease. This was well documented by de Faire (1974) in his study on death discordant twins where he found that the surviving partner of deceased twins with a diagnosis of coronary heart disease more often had symptoms and signs of this disease.

Suicides and to some extent accidents occur to a significantly higher extent among smokers. Out of 7 suicides in the monozygotic discordance groups 6 are found among the high exposed twins. If smoking is held to be unlikely as a causative factor for suicides the estimate of hypermortality is no doubt unduly influenced by the suicide rate.

As the major part of the data from the Swedish twin registry is based on questionnaire information and subsequent mortality follow-ups it has not been possible to study to any great extent physiological risk factors. Instead the emphasis has been put on behavioral and psycho-social variables as well as on genetic interactions.

The results clearly show that differences have been revealed for a large number of behavioral and psycho-social items also in very young people smoking only a few cigarettes a day. The smokers deviated from the nonsmokers not only with regard to behavioral and psycho-social data; they did so also with respect to some effect variables such as back disorders and continuous sick leave for long periods where the causal link to smoking seems remote. Further support for differences between smokers and nonsmokers was provided by very high hypermortality ratios for suicides. The associations between smoking and registration in an alcohol registry point in the same direction. The finding that several habitual risk factors as well as morbidity and mortality in the MET analysis were increased among nonsmokers if they had partners that were classified as present smokers indicates that smoking may be influenced by both genetic and social heredity.

The results fit a model in which nonsmokers and smokers are different in regard to a life-style parameter of which smoking is but one index others being alcohol drinking, drug abuse, psycho-social discord etc. It may be assumed that this parameter ranges from a "healthy" value at the one extreme to an "unhealthy" value at the other extreme. The concordant nonsmokers probably have values close to the first mentioned extreme and concordant smokers close to the other. Leaving the discordant smokers to occupy more undecided values in the middle of the range. If this construct variable is associated with disease which is highly probable it is obvious that nonsmokers in discordant pairs should experience ill health to a higher degree than concordant nonsmokers. Whether the basic explanation is predominantly genetic or environmental may be open for speculation. However from the analysis of the twin pairs it does seem that monozygotes as a rule fit the model better than dizygotes as demonstrated by better control of most habits.

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From the Department of Medicine University of Oulu Finland
(Head : Professor W J Kaipainen M D)

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cholesterol as a risk factor of IHD

cholesterol is one of the most thoroughly investigated risk factors included in all comprehensive works on the epidemiology in IHD. Following data on cholesterol have been recorded in USA (Serum cholesterol levels of adults National Center for Health Statistics). The cholesterol level increases in both men and women until, strictly the age of 40. In men older than that the increase then flattens off at a cholesterol value of approx. 230 mg/100 ml. In women the increase continues linearly until the age of 60 when the level is approx. 250 mg/100 ml. In USA 23 % of the men aged 35-44 have a cholesterol level above 250 mg/100 ml while a corresponding value among the women is 12.9 % of those aged 35-44. 28 % of those aged 45-54 and 49.7 % of those aged 55-64.

ghan Study (Karnel et al 1961) showed that the men aged 40-49 whose cholesterol value exceeded 244 mg/100 ml had a 3.4 times greater incidence than the men whose cholesterol value was below 244 mg/100 ml.

The corresponding risk for women was 2.4 times higher of the same study it has been concluded that the

in question the more significant is the cholesterol disease. In women aged over 50 the serum cholesterol is an important risk factor for coronary disease (Kannel et al 1966).

in the same study (Kannel et al 1971 a and b)
 serum cholesterol level is the risk factor most
 incidence of coronary disease. When the cholesterol
 level the ratio between observed and expected
 but when the cholesterol level was 300 mg/
 of threefold. These data pertain to men

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In several mass surveys the term ischemic heart disease has been replaced by the term coronary heart disease (CHD). In practice these two terms generally mean the same. For the present purposes the term ischemic heart disease was chosen as it refers to verified ischemia of the cardiac muscle regardless of the state of the coronary arteries or the symptoms of the subjects. The term coronary heart disease includes pathologico-anatomical changes in the coronary arteries which are severe enough to bring about a cardiac disease (Hurst 1974). In the following text however the term coronary heart disease has been used when referring to previous investigations.

According to the studies of Karvonen et al (1967 and 1970) the incidence and prognosis of IHD exhibit distinct regional differences in Finland. This observation is supported by the investigations of Rissanen (1972) and possibly also by the pathologico-anatomical investigations of children carried out by Pesonen et al (1975) though the latter studies did not directly pertain to IHD. The nature of IHD in each locality and the effect of risk factors on morbidity can be determined by well directed mass screening surveys. Thereby some basis is obtained for possible preventive measures.

For the above reasons it was considered expedient to conduct a mass screening survey in a typical northern Finnish municipality with a public health staff actively interested in such an investigation. A high percentage of participation could be expected which is one of the most important conditions for a successful realization of a project of this kind. Examination of women in comparison with men was also deemed important because several previous mass screening surveys had pertained to men.

The following risk factors of IHD were investigated: serum cholesterol and triglycerides, systolic and diastolic blood pressure, reduced glucose

INTRODUCTION

Finnish men have the highest mortality from ischemic heart disease (IHD) in the world (WHO 1967 and 1972 Miettinen 1969 and 1971 Pyörälä 1974). The cardiac mortality rate of Finnish women is also among the highest in the world (Epstein and Krueger 1969) being the highest in Scandinavia (Bolander 1971).

In addition to using statistics on the causes of death the occurrence of IHD can be investigated by mass surveys of the prevalence and incidence of IHD and the relationship of IHD to certain risk factors.

There are mass surveys in progress in different parts of the world they began as prevalence studies and continue as follow-up studies including repeated investigation of the subjects after a certain period which gives information on the incidence of IHD (Dawber et al 1957 Kannel et al 1962 Epstein et al 1965 Tibblin 1969 Welborn et al 1969).

Most of the mass surveys have been carried out on men while less interest has been directed to women. Recently, however, women have also been included among the series investigated (Bengtsson 1973 a Bengtsson et al 1973 b Hagerup 1973).

The best known among the Finnish mass surveys is the east west study (Karvonen et al 1967 Keys 1967 and 1970) which dealt with middle-aged men. There is also a Finnish investigation being carried out which pertains to both men and women and also covers localities in Northern Finland (Pyörälä et al 1973 Reunanen et al 1973). Comprehensive studies of the return to work by myocardial infarction patients (Vuopala 1972) and their rehabilitation (Palatsi 1976) have also been made in Northern Finland. Yet the data on the epidemiology of IHD

Serum cholesterol as a risk factor of IHD

Cholesterol is one of the most thoroughly investigated risk factors and is included in all comprehensive works on the epidemiology in IHD. The following data on cholesterol have been recorded in USA (Serum cholesterol levels of adults National Center for Health Statistics 1967). The cholesterol level increases in both men and women until approximately the age of 40. In men older than that the increase then levels off at a cholesterol value of approx 230 mg/100 ml. In women the increase continues linearly until the age of 60 when the level is approx 260 mg/100 ml. In USA 23 % of the men aged 35-64 have a cholesterol level over 259 mg/100 ml while a corresponding value among the women has been obtained for 12.9 % of those aged 35-44, 28 % of those aged 45-54 and 49.7 % of those aged 55-64.

The Framingham Study (Kannel et al 1961) showed that the men aged 40-59 whose cholesterol value exceeded 244 mg/100 ml had a 3.4 times higher CHD incidence than the men whose cholesterol value was below 210 mg/100 ml. The corresponding risk for women was 2.4 times higher.

On the basis of the same study it has been concluded that the younger the subject in question the more significant is the cholesterol level for coronary disease. In women aged over 50 the serum cholesterol level has no significance for coronary disease (Kannel et al 1966).

The 16-year report in the same study (Kannel et al 1971 a and b) further shows that serum cholesterol level is the risk factor most clearly affecting the incidence of coronary disease. When the cholesterol level was 200 mg/100 ml the ratio between observed and expected cases of new CHD was 0.7 but when the cholesterol level was 300 mg/100 ml this ratio was 2.1 or threefold. These data pertain to men.

tolerance obesity and overweight smoking and occupation The relationship of each risk factor to IHD was examined using certain ECG changes as the criteria of IHD

REVIEW OF THE LITERATURE

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aged 30 - 49

These are even more recent data on the Framingham series (Kannel and Castelli 1972) which show that high cholesterol values are associated with an increased risk of CHD in women aged below 50 just as they are in men. In women aged over 50 however triglycerides are a more significant risk factor.

On the basis of the Framingham findings Epstein (1967 b) predicted that 27 % of the men whose cholesterol level exceeded 260 mg/100 ml would develop coronary disease within 10 years.

The Albany study (Doyle et al 1959) of men aged 39 - 55 showed that the subjects with cholesterol below 200 mg/100 ml have an annual CHD incidence of 6.0/1000 ml while those with a cholesterol level higher than 275 mg/100 ml have a threefold CHD incidence or 19.0/1000.

In their work carried out in Minnesota Keys et al (1963) noted that the men aged 45 - 55 whose cholesterol exceeded 269 mg/100 ml had a 5.5 times higher CHD incidence than those whose cholesterol was below 200 mg/100 ml. In Evans County Georgia (McDonough et al 1965) the CHD prevalence was 2.2 times higher among the white men aged 40 - 74 whose cholesterol exceeded 250 mg/100 ml than among those whose cholesterol was below that value.

An investigation of white men in Los Angeles (Chapman and Massey 1964) revealed a 4.5 times higher frequency of myocardial infarction in those whose cholesterol was in the range 270 - 389 mg/100 ml than in those whose cholesterol was below 210 mg/100 ml. Subsequent results of the same investigation (Chapman et al 1971) show that the incidence of myocardial infarction becomes twofold in the cholesterol group of 270 - 389 mg/100 ml compared with the group below 210 /100 ml. The dependence between cholesterol and coronary disease has also been noted

The Busselton study carried out in Australia (Welborn et al 1969) showed that serum cholesterol, elevated blood pressure and declined glucose tolerance were all significantly and independently of each other associated with coronary disease in both sexes. The risk ratio for each approximately twofold.

The London study conducted by Morris et al (1966) revealed nearly 4 times more new cases of CHD in the highest cholesterol quintile than in the lowest quintile over a follow-up period of 5 years.

In a follow-up study of men in Oslo (Westlund and Nicolaysen 1966) the incidence of new coronary cases was 40.2/1000/year among those with a cholesterol value over 400 mg/100 ml but only 2.6/1000/year among those whose cholesterol value was below 200 mg/100 ml.

The Stockholm study by Carlson and Böttiger (1972) revealed a very low frequency of coronary disease but there was still a correlation between cholesterol and the incidence of coronary disease. In the Gothenburg study (Bangtsson et al 1973 a) on women on the other hand the cholesterol values of a myocardial infarction group, an angina pectoris group and a coronary ECG group did not differ from the control values.

The Finnish east-west investigation (Kavonen et al 1970) revealed a clear correlation between cholesterol on the one hand and myocardial infarction and sudden deaths on the other over a follow-up period of 5 years. The risk was 4 times higher in the highest quintile than in the lowest.

In this review Stanier (1973) concludes that as the cholesterol level rises the risk for atherosclerosis disease increases and that this dependence appears in all age groups at least from young adulthood through middle age. According to Stanier a cholesterol value below 200 mg/100 ml is normal, 200-249 mg/100 ml is border line and 250 mg/100 ml is pathological for Americans aged over 30. He

further postulates that people with a cholesterol value over 250 mg/100 ml have a twofold risk of developing premature coronary heart disease compared with those with a cholesterol value below that limit. Summarizing the research on coronary disease and cholesterol, Stamler maintains that the serum cholesterol level is the best simple measure for estimating the risk of atherosclerotic disease, particularly coronary disease.

In Ireland, Mulcahy et al. (1969) investigated 400 men aged under 60 suffering from coronary disease, finding high cholesterol, declined glucose tolerance, hypertension and smoking to be risk factors of CHD.

Nikkilä (1972) points out that the risk for coronary disease correlates strongly with the serum cholesterol level and becomes 3- to 5 fold within the range 200 - 300 mg/100 ml. The probability of coronary disease increases progressively the more the higher the cholesterol level, but the correlation apparently weakens beyond the age of 60.

Both cross-sectional and longitudinal studies have hence given rise to quite a unanimous agreement that serum cholesterol is a significant risk factor of IHD for both sexes, at least in younger and middle age, while women over 50 no longer display any clear correlation.

Serum triglycerides as a risk factor of IHD

The information available on triglycerides as a risk factor IHD is markedly less abundant than that on cholesterol, because triglyceride assays were included in the mass investigations later than the cholesterol assays.

According to the most recent Framingham reports (Kannel and Castelli 1972), triglycerides are a more significant risk factor than cholesterol in women over 50, while in women aged under 50 cholesterol is more important, as it also is in men within all the age classes studied (30

62 years)

Of the other studies made in USA at least the investigation conducted by Brown (1969) and Rosenman et al (1970) revealed a correlation between triglycerides and IHD

In Carlson and Böttiger's study (1972) in which over 3000 men were followed up for 9 years the frequency of IHD (myocardial infarctions + sudden deaths) increased linearly along with the rise of triglyceride and cholesterol levels. The authors point out that plasma triglycerides and cholesterol are risk factors for IHD independent of each other and a combined elevation of these two plasma lipids carries the highest risk for IHD.

The Gothenburg study of women (Bengtsson et al 1973 a) revealed a significantly higher triglyceride level in the women with myocardial infarction and those with coronary ECG than in the control group. The women with angina pectoris however had no difference in triglycerides compared with the control group.

Kitter-Hauge and Enge (1973) performed selective coronary angiography on 71 patients of whom 46 had had myocardial infarction and 25 had angina pectoris. This work established no correlation between the degree of obstruction of the coronary vessels and serum triglycerides or cholesterol.

In this review Stamler (1973) discusses his own epidemiological studies stating thus: Fasting serum triglycerides are not superior predictors of risk. Earlier claims of this kind based on preliminary or unsatisfactory data have not withstood the test of time.

In the survey of policemen carried out by Pyörälä et al (1976) triglycerides correlated with the risk of myocardial infarction together with systolic and diastolic hypertension, plasma cholesterol, relative weight and smoking over the 5-year follow-up but in a multivariate analysis triglycerides and diastolic hypertension were elimi-

nated from among the risk factors. The combination of high cholesterol and high triglycerides turned out as a prominent factor for the risk of myocardial infarction even in that work.

The significance of triglycerides as an independent risk factor of IHD so far remains unclear, but there is probably no doubt that a high triglyceride level combined with high cholesterol correlates strongly with ischemic heart disease.

Hypertonia as a risk factor of IHD

The correlation between hypertonia and IHD has been studied widely, especially in men, the corresponding data for women being much less frequent.

It is unanimously agreed at the present time that systolic and diastolic blood pressure are risk factors of IHD (Kannel et al 1961, Epstein 1965, Stamler et al 1966, Paul 1971, Gordon and Kannel 1972).

According to the Framingham study, the incidence of atherosclerotic heart disease in men with blood pressures ranging within 140/90 - 160/95 was 2.4-fold compared with the average over a follow-up period of 4 years (Opstad 1966).

According to the same study (Kannel et al 1961), the 40- to 59 year old men in the upper 5 per cent level of blood pressure had a 2.6 fold CHD incidence compared with the population at risk, while the women of the same age had a 6 fold risk.

According to an early Tecumseh report (Epstein et al 1965), elevated blood pressure is clearly associated with increased CHD prevalence.

The western collaborative study (Roseman et al 1966) showed the CHD incidence to be 3.6 times higher in the subjects with a diastolic blood pressure over 94 mmHg than in those with a diastolic pressure below that value.

In the Evans County study (McDonough et al 1965) the CHD prevalence was 2.3 times higher in the subjects whose blood pressure exceeded 160/90 than in those with lower values.

Cotton et al (1972) compared 91 men aged under 55 suffering from coronary disease and 98 healthy men finding out that the following factors dominated in the coronary patients: diastolic hypertension, arcus, baldness, xanthelasma, a family history of hypertension, past smoking habits and hyperlipemia. Of these factors, diastolic hypertension emerged as such the most important.

Mulcahy et al (1967) examined 100 women with coronary disease (35 had angina pectoris, 21 acute coronary insufficiency and 44 myocardial infarction). Hypertension, cigarette smoking and hypercholesterolemia were found to be risk factors. Cigarette smoking and hypertension were recorded together or separately for 80-90% of the patients. These findings gave rise to the conclusion that correct control of hypertension and cessation of smoking could reduce even dramatically the CHD incidence in middle-aged women.

According to Stamler (1964) both morbidity in myocardial infarction and coronary mortality correlate with diastolic hypertension. In the work on DuPont employees (Pell and D'Alonzo 1961) hypertensive subjects were also found to have an increased frequency of myocardial infarctions. According to the data of the Health Insurance Plan of Greater New York (HIP) (Weinblatt et al 1968) both mortality from myocardial infarction and recidivous infarctions were more common among hypertensive subjects than among normotensive ones. Chapman and Massey (1964) however noted no correlation between myocardial infarction and elevated blood pressure in their follow-up study of men aged below 50. A study based on the autopsy material of the International Atherosclerosis Project (McGill 1968) on the other hand revealed a significant correlation between hypertension and the severity of atherosclerosis.

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In the Busselton study carried out in Australia (Welborn et al 1969) hypertension was found to be a risk factor of CHD in both men and women. The study of women by Swedish Bengtsson (1973 b) revealed significantly more cases with hypertonia and a family history of hypertension in the group with myocardial infarction than in the control group.

In the Finnish east west study (Karvonen et al 1970) hypertonia was found to correlate with CHD incidence in men aged 40 - 59 years over a follow-up period of 5 years.

Simborg (1970) states that even mild hypertonia is associated with increased CHD incidence. He further points out that hypertonia can now be treated effectively but it is not known whether the treatment has any reducing effect on CHD incidence.

Stamler (1973) similarly establishes hypertonia as a risk factor of CHD independent of other risk factors e.g. hypercholesterolemia or smoking.

Kannel (1974) concludes from his findings that hypertonia is the most general and potent contributor to cardiovascular mortality among all the risk factors. He also states that elevated pressure casual or basal labile or fixed systolic or diastolic at any age in either sex is a potent contributor to all forms of cardiovascular disease.

According to the current investigations both systolic and diastolic hypertension are thus among the most potent risk factors in both sexes.

Diabetes mellitus and glucose intolerance as risk factors of IHD

The reports on the prevalence of clinical coronary disease in diabetics vary from 26 to 42 % (Ostrand et al 1965 b Lai and Bahl 1967). These studies reveal the significant feature that IHD is common in both female and male diabetics.

Herman and Gorlin (1965) noted that studies among persons with unexplained or premature coronary artery disease reveal an unusually high number with preclinical diabetes : Autopsy studies of diabetics show significant coronary disease in 75 - 89 % of cases (Stears et al 1947 Marble 1955)

According to some investigations people with atherosclerotic disease have reduced glucose tolerance more often than the controls (Inter society commission for heart disease resources 1970 Stamler 1967 Stamler et al 1972 Stamler and Epstein 1972)

Stamler et al (1960) found the occurrence rate (prevalence and incidence) of CHD to be twice as high in diabetics as in nondiabetics

According to the Framingham study (Kannel et al 1967 a) the CHD incidence was 1.4 times as high in diabetics as in nondiabetics among the 30- to 59-year old men with no signs of CHD at the beginning of the study. The corresponding risk of female diabetics was 2.5-fold. The risk of death from CHD was 2.3 times higher in diabetic men and 5.7 times higher in diabetic women.

The Tecumseh study (Stamler and Epstein 1972) showed that asymptomatic hyperglycemia correlated with CHD prevalence in both men and women and was independent of e.g. cholesterol and hypertension. CHD incidence was also found to correlate with hyperglycemia.

In the study of DuPont Company (Stamler 1967 Stamler et al 1972 Stamler and Epstein 1972) which covered 73000 women and men aged 25 - 64 clinical diabetes was found to be an independent risk factor of CHD.

In the study of Peoples Gas Company in Chicago (Stamler et al 1972) it was found out that during 1965 - 1970 mortality from coronary disease was 42/1000 among hyperglycemic subjects and 16/1000 among normoglycemic ones. The same study also revealed a correlation between hyperglycemia and hypertension: the higher the blood sugar content was after an hour's loading, the higher were the systolic and diastolic blood pressure val

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In the study of Peoples Gas Company in Chicago (Stamler et al 1972) it was found out that during 1965-1970 mortality from coronary disease was 42/1000 among hyperglycemic subjects and 16/1000 among normoglycemic ones. The same study also revealed a correlation between hyperglycemia and hypertension: the higher the blood sugar content was after an hour's loading the higher were the systolic and diastolic blood pressure values.

Krotkiewski et al (1970) examined 61 diabetics (38 men 23 women age 19 - 49 yr) without any sign of cardiovascular disease in Warsaw. Water's step test showed ST/T changes suggestive of latent coronary disease in 19.5 % of the patients (women 30.4 % men 13.1 %). No differences connected with the age of the patients, the duration of diabetes or the therapy given for it were seen.

Ostrovskaya-Tsarfis et al (1972) examined 5107 middle-aged men from the Moscow area for diminished glucose tolerance and noted that the incidence of disturbed tolerance to carbohydrates was 43.6 % including diabetes mellitus 3.5 %. The incidence is statistically significantly associated with the level of cholesterol and not associated with the level of triglycerides and total lipids of the blood serum. Disturbed tolerance to carbohydrates in middle-aged men is one of the independent factors of the risk for the ischemic disease of the heart. The significance of this factor grows when it is combined with marked hypercholesterolaemia.

In the Australian Busselton study (Welborn et al 1969) high serum cholesterol, elevated blood pressure and diminished glucose tolerance were significantly and independently of each other associated with coronary disease in both sexes. The risk was about twofold.

In the investigation carried out by Bengtsson et al (1973 c) a group with myocardial infarction turned out to have significantly more manifest diabetes than the control group. No corresponding difference appeared in the angina pectoris group or the group with pathological ECG. Nor did an i.v. glucose injection reveal any significant differences between the different groups. This investigation pertained to women.

Epstein (1967 a) postulates that there is a correlation between hyperglycemia and myocardial infarction. Hyperglycemia is somehow associated with hyperlipemia, positive calorie balance, hypertonia and

smoking but it remains to be elucidated whether hyperglycemia is an independent risk factor of IHD

In his cross sectional study of policemen in Helsinki Lehtovirta (1973) noted that both manifest diabetes and reduced glucose tolerance are closely correlated with obesity. The same phenomenon was discussed by Miettinen (1971) who pointed out that adult age diabetes correlates with obesity and hypertriglyceridemia.

In the 5-year follow-up of the Helsinki policemen (Pyörälä et al 1976) neither diabetes nor reduced glucose tolerance emerged as independent risk factors or as risk factors of myocardial infarction in a multivariate analysis.

Manifest diabetes is hence generally considered a risk factor of IHD while the significance of reduced glucose tolerance as an independent risk factor varies in both cross sectional and longitudinal studies. In any case diabetes and reduced glucose tolerance correlate with obesity and hyperlipidemia which combination in turn is a significant risk factor of IHD.

Overweight and obesity as risk factors of IHD

The relationship of overweight and obesity to ischemic heart disease has been investigated in several mass surveys.

In an early Framingham report (Dawber et al 1957) a correlation between obesity and atherosclerotic heart disease was noted in men aged 45.

In another early report of the same investigation (Kannel et al 1962) an increased CHD incidence was associated with cases where overweight exceeded 30 % but not with those where the percentage was smaller. Of the risk factors examined obesity had the poorest correlation with coronary disease.

Krotkiewski et al (1970) examined 61 diabetics (38 men 23 women age 19 - 49 yr) without any sign of cardiovascular disease in Warsaw. Mater's step test showed ST/T changes suggestive of latent coronary disease in 19.6 % of the patients (women 30.4 % men 13.1 %). No differences connected with the age of the patients, the duration of diabetes or the therapy given for it were seen.

Ostrovskaya-Tsarfis et al (1972) examined 5107 middle-aged men from the Moscow area for diminished glucose tolerance and noted that the incidence of disturbed tolerance to carbohydrates was 43.5 % including diabetes mellitus 3.5 %. The incidence is statistically significantly associated with the level of cholesterol and not associated with the level of triglycerides and total lipids of the blood serum. Disturbed tolerance to carbohydrates in middle-aged men is one of the independent factors of the risk for the ischemic disease of the heart. The significance of this factor grows when it is combined with marked hypercholesterolaemia.

In the Australian Busselton study (Welborn et al 1969) high serum cholesterol, elevated blood pressure and diminished glucose tolerance were significantly and independently of each other associated with coronary disease in both sexes. The risk was about twofold.

In the investigation carried out by Bengtsson et al (1973 c) a group with myocardial infarction turned out to have significantly more manifest diabetes than the control group. No corresponding difference appeared in the angina pectoris group or the group with pathological ECG. Nor did an i.v. glucose injection reveal any significant differences between the different groups. This investigation pertained to women.

Epstein (1967 a) postulates that there is a correlation between hyperglycemia and myocardial infarction. Hyperglycemia is somehow associated with hyperlipemia, positive calorie balance, hypertonia and

infarction and obesity but found no significant correlation between them

The Australian Busselton study (Welborn et al 1969) revealed no significant correlation between obesity and coronary disease in either sex

The studies by Keys et al (1972) showed a clearly increased incidence of coronary disease relative to overweight and obesity in men aged 40-59 in USA and Southern Europe but not in Northern Europe. The multivariate analysis of the same study showed that neither relative nor obesity were independent risk factors of imminent CHD when age, blood pressure, cholesterol and smoking were comparable. Relative weight and obesity correlated with blood pressure and serum cholesterol. Their correlation with smoking was negative: the smokers were thinner than the non-smokers. This investigation also included Finnish men (the East West study).

In the investigation of policemen aged 30-59 in Helsinki conducted by Pyörälä et al (1976) obesity had a prognostic value for the risk of myocardial infarction independent of blood pressure and blood lipids; this finding was made in the older age-groups but not in the younger. The multivariate analysis of the same study established smoking, systolic hypertension and plasma cholesterol along with relative weight as risk factors of myocardial infarction.

Hietinen (1971) maintains in his review of diabetes that if obesity is associated with diabetes of adult onset, concurrent hypertriglyceridemia is very common. It has also been noted that the plasma insulin content of obese subjects is often increased although their glucose tolerance may be normal (Mikkilä et al 1965; Chiles & Tzagournis 1970).

Both cross sectional and longitudinal studies have thus shown correlations between overweight and obesity on the one hand and different

According to two subsequent Framingham reports (Dawber et al 1963 Kannel et al 1967 b) CHD incidence increases along with overweight but no correlation between overweight and myocardial infarction was noted in any age class of either sex. Angina pectoris and overweight however have a strong positive relationship in both men and women. Furthermore overweight was found to correlate with sudden deaths due to arrhythmias.

In his extensive survey Simborg (1970) postulates that there is a weak correlation between overweight and total CHD incidence but that no correlation probably exists between overweight and myocardial infarction or overweight and sudden deaths due to myocardial infarction. Obesity predisposes to angina and sudden death from arrhythmia but does not predispose to myocardial infarction.

In the Tecumseh study (Epstein et al 1965) a 1.7 fold CHD incidence was noted among those in the uppermost quintile of relative weight compared with the other men or women.

In their prospective study Stamler et al (1960) noted a twofold CHD incidence in the subjects whose relative weight exceeded 113 compared with those who had a corresponding value below 100. In that study CHD included myocardial infarction, sudden death, coronary insufficiency and congestive cardiac insufficiency due to coronary disease.

The investigation of businessmen in Los Angeles (Chapman and Massey 1964) on the other hand revealed no correlation between overweight and CHD during a 16 year follow-up.

A similar result was also obtained in a corresponding study carried out in Chicago (Paul et al 1963). These investigators also measured the thickness of skinfolds which correlated positively with CHD incidence. Both of these works included myocardial infarction and angina pectoris under the label of CHD.

Gertler and White (1954) and Frank et al (1966) examined myocardia

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Both cross-sectional and longitudinal studies have thus shown correlations between overweight and obesity on the one hand and different

manifestations of IHD on the other but the findings are somewhat contradictory. The greatest significance of obesity as a risk factor of IHD probably lies in the fact that it is closely associated with hypertension, hyperlipidemia and diabetes.

Smoking as a risk factor of IHD

Cigarette smoking has turned out to be a significant risk factor of IHD particularly in young and middle-aged men (Doll and Hill 1964, Doyle et al 1964, Frank et al 1966, Hammond 1966, Hill and Wynder 1974). The cross-sectional studies at Tecumseh, USA (Epstein et al 1965) and Busselton, Australia (Welborn et al 1969) established no correlation between smoking and angina pectoris or ischemic ECG changes and the cross-sectional findings at Framingham also showed that angina pectoris is no more common among smokers than non-smokers (Dawber et al 1957). The correlation between smoking and IHD generally appears clearly in longitudinal studies only. In the National Co-operative Pooling Project, a follow-up study of men aged 30-59 showed that cigarettes rendered the incidence of myocardial infarction twofold independently of the other risk factors; the risk of sudden death was found to be 1.9 fold for those who smoked 10 cigarettes daily, 3.4 fold for those who smoked over 20 cigarettes daily (Inter Society Commission for Heart Disease Resources 1970).

Cigarette smokers also have a shorter life-time after the appearance of coronary symptoms than non-smokers, and those who give up smoking have a lower incidence of myocardial infarction and death from CHD than those who continue smoking (Spain and Bradess 1970).

Several follow-up studies (e.g. Morris et al 1966, Tibblin and Wilhelmsen 1970) have shown that cigarette smokers have a higher CHD incidence than non-smokers.

Rose (1976) estimated that the men who smoke 15 cigarettes daily have a twofold CHD risk compared with non-smokers and the risk of those who smoke 40 cigarettes is a 12.5-fold

Cigarette smoking has been found to increase the incidence of angina pectoris in some studies (e.g. Shapiro et al 1969) while in some others the incidence of angina pectoris does not correlate with cigarette smoking (e.g. Tibblin and Wilhelmsen 1970). Cigarette smoking has been found to diminish the exercise tolerance of angina pectoris patients (Aronow et al 1968, Aronow and Rokaw 1971, Aronow and Swanson 1969 a). Cigarettes with a high nicotine content give rise to chest pain during exercise more easily than cigarettes with a low nicotine content (Aronow et al 1968) which in turn produce chest pain upon exercise in angina pectoris patients more easily than cigarettes containing no nicotine (Aronow and Swanson 1969 b).

The cross sectional study of nearly 20000 middle-aged (40-64 yr) British men carried out by Reid et al (1976) showed a positive correlation for angina pectoris and anamnestic myocardial infarction on the one hand and smoking on the other and this association was independent of e.g. high cholesterol or hypertension. The follow-up of this series (5 yr) revealed a correlation between smoking and coronary mortality which was also independent of other risk factors.

The Framingham Study showed (Inter Society Commission for Heart Disease Resources 1970) that if abundant cigarette smoking (20 cigarettes/day) was accompanied by high cholesterol (over 7.8 mmol/l) and elevated diastolic blood pressure (over 105 mmHg) the risk of myocardial infarction or CHD death became 8.5-fold over the 10-year follow-up. Several other surveys (e.g. Wilhelmsen et al 1973) have also indicated that the combination of hypertension, high cholesterol and cigarette smoking among men are highly prognostic of myocardial infarction and sudden death.

Werkö (1976) points out in his review that if smoking is associated with high cholesterol or high blood pressure or both the risk of coronary death becomes about fourfold. At the same time Werkö makes the conclusion that no single risk factor is of any greater importance by itself.

The Framingham report on smoking after 18-year follow-up (Gordon et al 1974) shows that non-smokers pipe- or cigar-smokers and those who smoked 10 cigarettes or less daily had lower CHD mortality than those who smoked more than 10 cigarettes daily. This finding applied to men. The men who smoked at the beginning of the investigation but then gave up smoking had only a half of the number of CHD attacks suffered by the men who went on smoking.

In her follow-up study made in Norway (about 8000 middle-aged women and 7000 men) Zeiner-Henriksen (1976) examined the relationship between CHD mortality and smoking over 11 years. The highest mortality was recorded for the cigarette-smoking men and the mixed smokers (both cigarettes and pipe) in both urban and rural populations. It was also noted that rural men had the same CHD mortality regardless of whether they smoked pipe/cigar or cigarettes. Some other investigations have also shown a positive correlation between CHD mortality or incidence and pipe/cigar smoking (Inter Society Commission for Heart Disease Resources 1970 Shapiro et al 1969).

The same study by Zeiner Henriksen revealed equal CHD mortality among female non-smokers and smokers in both urban and rural populations.

Bengtsson (1973 d) found no differences in smoking among women with angina pectoris and women with coronary ECG. The group of women with myocardial infarction however contained significantly more smokers than non smokers.

The east-west study showed CHD mortality to be considerably higher among both ex smokers and smokers than male non-smokers (Punsar and Pyörälä 1971 Punsar 1970)

Ball and Turner (1974) point out that 52000 Britons die annually for causes directly dependent on smoking and that about half of these deaths are due to cardiovascular diseases mainly CHD. Of the consumption of cigarettes were reduced by 20 % there would be 8000 deaths fewer in 1984. This would reduce the number of coronary deaths more than any other method known.

Karvonen estimated (1972) that 1000-1800 working age men die a coronary death in Finland every year owing to cigarette smoking.

The correlation between cigarette smoking and IHD is uncertain in cross-sectional studies while longitudinal studies have conclusively shown cigarette smoking to increase the sudden deaths of men due to coronary disease. Smoking also increases the CHD incidence among men though the effect of smoking on angina pectoris remains open to speculation. It seems however that smoking reduced the exercise tolerance of angina pectoris patients. There is also some evidence indicating that pipe/cigar smoking would similarly correlate with CHD mortality among men. As regards women there is not yet sufficient evidence of any correlation between CHD and smoking.

The combination of smoking, hypertension and high cholesterol in men has been found to be the most serious risk factor of myocardial infarction and sudden coronary deaths.

Occupation as a risk factor of IHD

There is not much literature dealing with the correlation between IHD and occupation. There are however several studies on IHD and physical activity and inactivity involved in the occupation. Most of these studies have shown higher CHD mortality and incidence in inactive

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In her follow-up study made in Norway (about 8000 middle-aged women and 7000 men) Zeiner Henriksen (1976) examined the relationship between CHD mortality and smoking over 6 years. The highest mortality was recorded for the cigarette-smoking men and the mixed smokers (both cigarettes and pipe) in both urban and rural populations. It was also noted that rural men had the same CHD mortality regardless of whether they smoked pipe/cigar or cigarettes. Some other investigations have also shown a positive correlation between CHD mortality or incidence and pipe/cigar smoking (Inter Society Commission for Heart Disease Resources 1970 Shapiro et al 1969).

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Karvonen et al (1972) noted a higher CHD incidence among the lumberjacks in Ilomantsi than the other men of the same locality over a follow-up period of 10 years

Pyörälä (1972) examined the association between CHD and physical activity on the basis of epidemiological investigations made in different parts of the world. In these works physical activity has frequently been expressed in terms of occupation. Pyörälä concludes that physical inactivity is accompanied by an increased risk of coronary disease. According to him this risk is independent of other risk factors such as hypertension, cholesterol level or obesity.

So far the correlation between IHD and occupation is vague for both sexes. Some studies have shown that agricultural workers have less coronary disease than people doing lighter work. Finnish lumberjacks, however, were found to have a higher CHD incidence than the other men covered in the investigations.

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In a survey of kibbutz workers in Israel (Brunner & Manelis 1971) agricultural workers were found to have a lower CHD incidence than ones doing light work this applied to both women and men The same results also showed that agricultural workers had a lower incidence of angina pectoris myocardial infarction and coronary deaths than people with light jobs

The Evans County Study (Cassel et al 1971) showed that agricultural workers had a CHD incidence which was only about half of the corresponding incidence of people holding other occupations and they were also found to have lower CHD mortality

Zeiner Henriksen (1976) examined CHD mortality in different occupational groups 1) workers in agriculture or forestry and fishermen 2) workers in commerce and industry and service occupations 3) of officials administrators etc The investigation covered about 7000 men and 8000 women During the follow-up of six years CHD mortality was highest in group 2 and lowest in group 1 It was possible that the people in group 1 had the most active occupation physically This finding pertained to men no corresponding result for women is reported

Bengtsson (1973 c) found a correlation between physically inactive occupation and myocardial infarction in a female series including 47 women who had suffered a myocardial infarction He did not note any significant difference in socioeconomic status between the IHD women and the control women (Bengtsson 1973 e)

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MATERIAL

The investigation was carried out at Haapavesi which is a typical Northern Finnish municipality. It is located in Northern Ostrobothnia approx. 140 km south of Oulu. In 1971 when the investigation was made there were about 7500 inhabitants at Haapavesi with 50-60 % working in agriculture and forestry and fairly equal portions of the remaining group in commerce, transportation and services.

The project was carried out during five weeks in April-May 1971. The examinations were made in the Haapavesi Local Hospital. All the men and women living at Haapavesi who were 40-59 years old according to the census list made on Jan. 1, 1970 were invited to the examination by a personal letter. Altogether 1686 invitations were sent.

The census list for 1971 which was completed immediately after the beginning of the investigation showed that 36 of those invited had either died or moved away from Haapavesi during 1970. Conversely 50 people had moved there and 24 of them came to be examined.

Thus there were altogether 1710 people aged 40-59 at Haapavesi at the time of the investigation and 1554 of them participated in it. The percentage of participation was hence 90.9 % 793 women and 761 men were examined.

The series of subjects is described in greater detail in the chapter on results and in the discussion.

PURPOSE OF THE PRESENT STUDY

The purpose was

- to study the prevalence of IHD among middle aged rural men and women
to examine the correlation of certain risk factors as well as smoking
and occupation to IHD
- to find out whether the men and women differ from each other in the
occurrence and significance of these factors

METHODS

Laboratory studies

The subjects came to be examined after 12-15 hours fasting. First a venous blood sample was drawn for cholesterol and triglyceride assays (Pearson et al 1953, Royer and Howard 1969) and a capillary blood sample for a determination of blood sugar (Hyvärinen and Nikkilä 1962). Immediately thereafter the subjects were given sugar solution (Glukodyn^R) 1 g/kg and after two hours a new capillary blood sample was drawn for determining the 2 hour blood sugar.

The blood sugar assays were made immediately in the laboratory of the local hospital; the cholesterol and triglyceride measurements were performed later on deep-frozen sera in the laboratory of the Oulu Deaconess Institute.

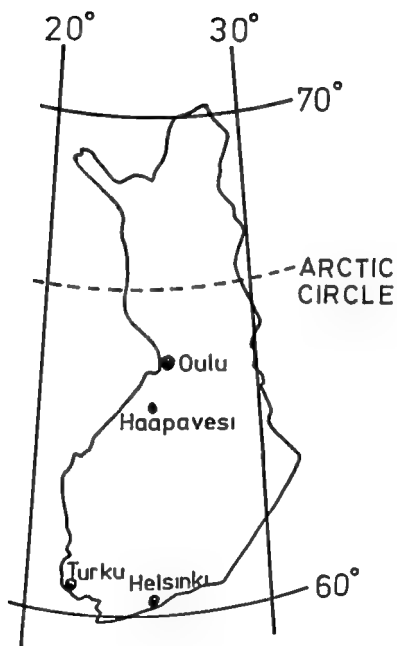
Anthropomorphic measurements

The height and the weight were measured in light underclothing without shoes. The height was recorded with an accuracy of 0.5 cm, the weight with an accuracy of 0.5 kg.

The height and weight readings yielded the value of overweight when the weight (W) was divided by the height (H)

$$\frac{W}{H} \quad \text{weight/height index (Khosla and Lowe 1967)}$$

Although this index is not the best of the indexes constructed on the basis of the height and weight, it is simple and was therefore considered suitable for this investigation, particularly as obesity was also verified by skinfold measurements. The triceps and the subscapular skinfolds were measured according to standardized instructions (Rose and Blackburn 1968). The results were recorded with an accuracy of 1 mm.



version of the Minnesota Code (Rose and Blackburn 1968) was used

The subjects examined were divided into two groups on the basis of the ECG

I ECG-positive (pathological ECG)

II ECG-negative

The subjects in whom one or several of the following changes were noted were ECG-positive

I Minnesota code 1 1 ("major Q")

II Minnesota code 4 1 5 1 5 2 ("major ST/T depression")

III Minnesota code 6 1 (complete A V block) 7 1 (complete left bundle branch block) and 8 3 (atrial fibrillation)

The others were ECG-negative

Minnesota codes used as criteria of pathological ECG

1 1 1 Q/R amplitude ratio 1/3 or more plus Q duration 0 03 sec or more in any of leads I II V2 3 4 5 6

1 1 2 Q duration 0 04 sec or more in any of leads I II V1 2 3 4 5 6

1 1 3 Q duration 0 04 sec or more plus R amplitude of 3 mm or more in lead aVL

1 1 4 Q duration 0 05 sec or more in lead III plus any r wave of at least 1 0 mm amplitude in aVF

1 1 5 Q duration 0 05 sec or more in lead aVF

1 1 6 QS patterns when R wave is present in adjacent lead to the right on the chest in any of leads V2 3 4 5 6

Blood pressure

The blood pressure was recorded from the right upper extremity in a sitting position according to the WHO instructions (Rose and Blackburn 1968). Diastolic pressures were taken at the fifth phase (i.e. point of disappearing of all sounds). A mercury manometer with a 12 x 25 cm cuff was used. The reading was taken with an accuracy of 5 mmHg.

The blood pressures were recorded by three local public health nurses on alternate days. Blood pressure measurements are part of their daily routine which has given them a fairly wide experience. Despite this the manner of recording the blood pressure was controlled for each of the three nurses before starting the examinations so that the technique of measurement would be as identical as possible in all cases.

Electrocardiograms

The apparatus employed was Hewlett Packard's ECG-phonosystem 1514 A which meets the criteria set for ECG equipment (American Heart Association 1967, Krikler and Macfarlane 1974). Paper speed was 50 mm/s and calibration 1 mV/cm. ECG was recorded 1.5-2 hours after the glucose administration according to the WHO instructions (Rose and Blackburn 1968). The leads I, II, III, aVR, aVL, aVF, V1, V6 were used. All the ECG recordings and interpretations were made by the author.

Pathological ECG

There are several ECG classifications now available which are suited to mass surveys (Blackburn et al 1960, Astrand et al 1967, Punsar et al 1968, Blackburn 1969). In interpreting the present ECGs the WHO

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1 1-4 Q duration 0 05 sec or more in lead III plus any Q wave of at least 1 0 mm amplitude in aVF

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- 1-1-7 QS pattern in all of leads V1-V4 V1-V5 or V1-V6
- 4 1 S T-J depression 1 0 mm or more and S-T segment horizontal or downward sloping in any of leads I II aVL aVF V2 3 4 5 6 (requires a T-wave code in 5)
- 5-1 T amplitude negative minus 5 mm or more in any of leads I II V2 3, 4 5 6 or in lead aVL when R amplitude is 5 mm or more or in lead aVF when QRS is mainly upright
- 5-2 T amplitude negative or diphasic (positive-negative or negative-positive type) with negative phase at least minus 1 0 mm but not as deep as minus 5 mm in any of leads I II V2 3 4 5 II or in lead aVL when R amplitude is 5 mm or more or in lead aVF when QRS is mainly upright
- 6 1 Complete (third degree) A V block (permanent or intermittent) in any lead
- 7-1 Complete left bundle branch block
- 8-3 Atrial fibrillation

Comparison of risk factors and pathological ECG

The correlation of the following factors to pathological ECG was investigated cholesterol triglycerides systolic and diastolic blood pressure declined glucose tolerance overweight and obesity as well as smoking and occupation

The comparison was accomplished by dividing the subjects into five equally large groups (quintiles) on the basis of each risk factor and finding out the proportion of ECG-positive subjects in each quintile The comparison for smoking and occupation was carried out differently

If we take cholesterol as an example quintile 1 consisted of individuals having the lowest 20 % of the values quintile 2 comprised the following 20 % etc Quintile 5 thus represents the subjects with the highest cholesterol values

The same procedure was used for the other risk factors the glucose quintiles were based on the 2-hour glucose value and all those with verified manifest diabetes; who could not take the 2-hour glucose tolerance test were placed in quintile 5 The overweight quintiles were constructed on the basis of the weight/height index the subjects in quintile 1 had the lowest indexes those in quintile 5 the highest For the obesity quintiles the sum of the triceps and subscapular skinfolds was used with the individuals in quintile 1 having the smallest folds and those in quintile 5 the largest

Smoking and pathological ECG

The subjects were divided into three main groups on the basis of smoking smokers (1) ex smokers (2) and non-smokers (3) (Rose & Blackburn 1968) The proportions of pathological ECG values were compared in the different age-groups and the two sexes

The smokers were further divided into sub-groups on the basis of the quantity and quality of the tobacco consumed and the proportions of ECG-positive and ECG-negative subjects in the two sexes were compared in these sub-groups

Occupation and pathological ECG

The population examined was divided into the following occupational groups

- 1 = heavy manual work (farmer farmer's wife heavy household work with cattle raising lumbering)
- 2 = supervision stress job (teacher salesman shopkeeper physician)
- 3 = office work or light sedentary work (office clerk car transport service)
- 4 = standing work (shop assistant waiter/waitress nurse)
- 5 = household work (no cows)
- 6 = disabled pensioner

These occupational groups have been constructed on the basis of the classification proposed by Rauhala (1966) and an attempt has been made to adjust them to the local conditions

The proportions of pathological ECG values in the different age groups of the occupational categories were recorded for both sexes

Statistical methods

The t test (Woolf 1968 a) and a modified t-test (Woolf 1968 b) were used in comparing the means and the different ratios

The degree of significance were as follows

Almost significant (the probability was less than 5 % or $p < 0.05$)

Significant (less than 1 % or $p < 0.01$)

Highly significant (less than 0.1 % or $p < 0.001$)

Tree analysis

The correlations between the different risk factors and pathological ECG were examined with the help of the tree analysis or the AID (automatic interaction detector) method (Sonquist et al 1974)

The method is applicable when there is one dependent variable and at least one independent variable. The dependent variable must be

dichotomous or adaptable to an interval scale while the independent variable can be expressed with a nominal scale. Each independent variable comprises two or more classes.

The purpose of the method is to find the best combinations of independent variables making the differences between the observational items grouped on the basis of the combinations and the means of the dependent variable as great as possible. The method resembles the stepwise regression analysis in that it advances step by step starting from the independent variables which are most clearly dependent on the dependent variable. At each stage one group of observational items is divided into two. The process is continued until no group can be divided on the basis of the criteria any longer. The aim is to find the independent variables which correlate best with the dependent variable and their optimal combinations. The essential findings are expressed with a figure where the stepwise formation of the groups, the cutting points employed, the group means in the dependent variable and the size of the final groups are presented. The ultimate result yielded by this method is hence largely qualitative.

At the first stage all the items are dealt with as one group. The division into two groups is made by the independent variable and at the point which makes the sum of squares between the groups in the dependent variable as great as possible. In the group I which is to be divided the best point of intersection for each explaining variable is sought as follows:

Let TSS be the total variance of the variable to be explained in the material and TSSI the corresponding total variance in the group I to be divided (if $TSSI/TSS \geq P_1$ no attempt is made to divide group I) and let BSS be the variance between the two groups obtained by dividing

- 1 = heavy manual work (farmer farmer's wife heavy household work with cattle raising lumbering)
- 2 = supervision - stress job (teacher salesman shopkeeper physician)
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Tree analysis

The correlations between the different risk factors and pathological ECG were examined with the help of the tree analysis or the AID (automatic interaction detector) method (Sonquist et al 1974)

The method is applicable when there is one dependent variable and at least one independent variable. The dependent variable must be

MAXGP the greatest permitted number of final groups. The criterion ensures that no more groups are obtained than has been considered expedient for the investigation in advance.

RMIN the smallest permitted number of observational items in the group. If a group has fewer items than **2RMIN** it is no longer divided because at least one of the resulting groups would have fewer items than **RMIN**. This criterion prevents the differentiation of a few highly deviating items into a group of their own.

The condition of each criterion must be realized before the group can be divided. When the condition of **MAXGP** is fulfilled the process is terminated while the other conditions pertain to single groups.

It must further be made clear whether the independent variables are expressed with a nominal scale or an ordinal or some more complex scale. On the basis of this information the program treats the variables in different ways. The classes of a variable expressed with a nominal scale are set in an order on the basis of their means in the dependent variable but the order of the classes of the variables expressed with an ordinal or some other scale is retained. While searching for the cutting point the program then divides the observational items into two groups at each class boundary examining whether the criteria are realized and tabulating the result. It further tabulates the best cutting point for each variable. According to the **P2** criterion the best cutting points of the different variables are then examined for the point which is used to divide the group into two.

The final groups obtained as the result of the analysis can be different: small groups, explained groups and unexplained groups. The group is small whenever the number of items is too small for the **RMIN** criterion to allow further division. The group is explained when it is so homogenous that the **P1** criterion shows the variance to be so small as to allow no further division. The group is unexplained if it contains

group I The ratio BSS/TSSI is calculated for each informative variable at each point of intersection The greatest of these ratios corresponds to the best division which can be made by the variable to be explained The variable represented by the greatest ratio BSS/TSSI is chosen as the basis of the division The division is made if the ratio BSS/TSS thereby obtained $\geq P2$ It is further checked that the criteria MAXGP and RMIN are valid

This procedure guarantees the minimum variance of the dependent variable so far unexplained When the entire material has been divided into two groups like this the group with the greatest total sum of squares in the dependent variable is chosen to be examined next In other words the group as unhomogeneous as possible from the viewpoint of the dependent variable is chosen to be considered first because the variance within it is great and it can most likely be further divided into groups The division of this group yields again two groups which gives us a total of three groups The group with the greatest total sum of squares in the dependent variable is chosen to be considered next The process is then continued in the same way until none of the groups can be divided any longer according to the guiding criteria

The formation of groups is guided by four criteria

$P1$ = the proportion of the total sum of squares of the dependent variable which represents the minimum internal variance of the group required for the group to be divided The variation range of the criterion is $0.00001 \leq P1 \leq 1.0$ This criterion prevents the division of very homogenous groups

$P2$ = the proportion of the total sum of squares of the dependent variable which represents the minimum sum of squares between the groups obtained in the best division The variation range of the criterion is $0 < P2 <$

MAXGP the greatest permitted number of final groups. The criterion ensures that no more groups are obtained than has been considered expedient for the investigation in advance.

RMIN the smallest permitted number of observational items in the group. If a group has fewer items than **RMIN** it is no longer divided because at least one of the resulting groups would have fewer items than **RMIN**. This criterion prevents the differentiation of a few highly deviating items into a group of their own.

The condition of each criterion must be realized before the group can be divided. When the condition of **MAXGP** is fulfilled the process is terminated while the other conditions pertain to single groups.

It must further be made clear whether the independent variables are expressed with a nominal scale or an ordinal or some more complex scale. On the basis of this information the program treats the variables in different ways. The classes of a variable expressed with a nominal scale are set in an order on the basis of their means in the dependent variable but the order of the classes of the variables expressed with an ordinal or some other scale is retained. While searching for the cutting point the program then divides the observational items into two groups at each class boundary examining whether the criteria are realized and tabulating the result. It further tabulates the best cutting point for each variable. According to the **P2** criterion the best cutting points of the different variables are then examined for the point which is used to divide the group into two.

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variance but no independent variable can be used to divide it into two in such a way that a proportion of the variance required by the P2 criterion would be explained

RESULTS

Prevalence of pathological ECG

The total series included 319 ECG-positive subjects who account for 20.5 % 28 of them or 8.7 % of all the ECG-positive subjects belonged to group I ("major Q") 238 or 88.8 % to group II ("major ST/T depression") and 8 or 2.5 % to group III (complete A V block 1 complete left bundle branch block 5 and atrial fibrillation 2) Tables 1 and 2

There were 210 ECG-positive women who accounted for 26.5 % of the total female series (793) 6 of them or 0.8 % belonged to group I 202 or 25.5 % to group II and 2 or 0.3 % to group III Table 1

The ECG-positive men numbered 109 accounting for 14.3 % of the total male series (761) 22 or 2.9 % belonged to group I 81 or 10.6 % to group II and 6 or 0.8 % to group III

Pathological ECG in the different age groups, Tables 1 and 2, figure 1

In women the proportion of pathological ECG values increases evenly along with age there is a clear difference between the youngest and the oldest age group ($p < 0.001$)

In men the proportion of ECG-positive findings first increases clearly the difference between the youngest and the next oldest group is significant ($p < 0.01$) but from 45 years onwards the prevalence of positive ECG findings remains unchanged

A comparison of men and women shows that women have more ECG changes than men in the total series ($p < 0.001$) In each age group too more women have pathological ECG than men in the youngest and oldest age groups the difference is highly significant ($p < 0.001$) Pathological Q changes on the other hand are considerably more frequent among men than women ($p < 0.001$)

Table 1 ECG finding specified ; Women by age groups

I = major Q (Minn 1 1)

II = major ST/T depression (Minn 4 1 5 1 5 2)

III = others (Minn 6 1 7 1 8 3)

Age group	I	II	III	Total
40 - 44 233 subjects	0 = 0 %	42 = 18 0 %	0 = 0 %	42 = 18 0 %
45 - 49 197	0 = 0 %	46 = 23 4 %	1 = 0 5 %	47 = 23 9 %
50 - 54 197	1 = 0 5 %	55 = 27 9 %	0 = 0 %	56 = 28 4 %
55 - 59 166	5 = 3 0 %	59 = 35 5 %	1 = 0 6 %	65 = 39 1 %
Total 793	6 = 0 8 %	202 = 25 5 %	2 = 0 3 %	210 = 26 5 %

Table 2 ECG finding specified Men by age groups I II and III
as in table I

Age group	I	II	III	Total
40 44 229 subjects	3 1.3 %	13 5.7 %	1 = 0.4 %	17 7.4 %
45 49 202	5 2.5 %	25 12.4 %	2 1.0 %	32 15.9 %
50 54 161	6 3.7 %	22 13.7 %	1 = 0.6 %	29 18.0 %
55 59 169	11 4.7 %	21 12.4 %	2 1.2 %	34 18.3 %
Total 761	22 2.9 %	81 10.6 %	6 0.8 %	109 14.3 %

Figure 1 Pathological ECG in the different age groups

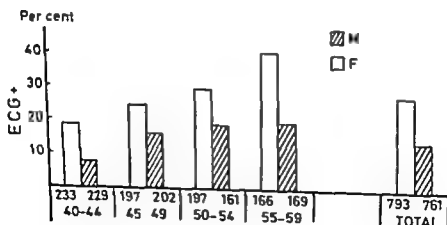


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45 49 197	0 = 0 %	46 = 23 4 %	1 = 0 5 %	47 23 9 %
50 54 197	1 = 0 5 %	55 = 27 9 %	0 = 0 %	56 28 4 %
55 59 166	5 = 3 0 %	59 = 35 5 %	1 = 0 6 %	65 = 39 1 %
Total 793	6 = 0 8 %	202 = 25 5 %	2 = 0 3 %	210 26 5 %

Summary

No correlation appears between cholesterol and pathological ECG

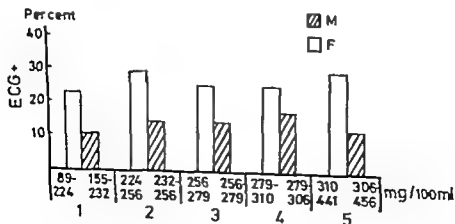
Table 3 Cholesterol means (mg/100 ml) in the different age groups

Age group	Women	SD	Men	SD
40-44	260	44.6	267	47.6
45-49	262	46.5	270	47.2
50-54	276	51.1	270	46.5
55-59	275	54.0	279	44.2
Total	268	49.3	271	46.5

Table 4 Cholesterol means (mg/100 ml) and pathological ECG

		SD
ECG-positive women	271	52.0
ECG-negative women	266	48.4
ECG-positive men	274	41.0
ECG-negative men	270	47.6

Figure 2 Cholesterol quintiles and pathological ECG



Serum cholesterol

The mean cholesterol in the entire series was 269 mg/100 ml. The mean for women was 268 mg/100 ml and that for men 271 mg/100 ml. The difference is not significant. Table 3

The mean cholesterol value increased along with increasing age in both sex groups (women $p < 0.01$ men $p < 0.05$). Table 3

The mean cholesterol for the ECG-positive women was 271 mg/100 ml and the mean for the ECG-negative women 266 mg/100 ml. The corresponding values for men were 274 and 270. The differences were not significant in either sex group. Table 4

The ECG-positive women who had a pathological Q wave (6) had a mean cholesterol values of 268 mg/100 ml. It does not differ from the mean cholesterol value of the ECG negative women (266 mg/100 ml).

Correspondingly the men with a pathological Q wave (22) had the same mean cholesterol value as all the ECG positive men i.e. 274 mg/100 ml. When this is compared with the cholesterol mean of the ECG-negative men (270 mg/100 ml) no significant difference can be seen.

Cholesterol quintiles and pathological ECG Figure 2

When the series is divided into quintiles according to the cholesterol values and the proportion of ECG positive subjects is examined in each quintile it appears that both men and women show a minor difference between the lowest and the highest quintile but this difference is not significant.

The three middle quintiles of men contained more ECG-positive subjects than either the first or the last but no significant differences appeared between any groups. ECG changes thus do not increase along with increasing cholesterol values in either sex though the variation of cholesterol values is 89-441 mg/100 ml in women and 155-456 mg/100 ml in men.

Summary

In the female series there is no correlation between triglycerides and pathological ECG

In the male series there is some correlation the men with a pathological Q wave in their ECG had higher serum triglyceride levels on an average than the ECG-negative men

Table 5 Triglyceride means (mmol/l) in the different age groups

Age group		Women		Men	
		Mean	SD	Mean	SD
40	44	1.1	0.5	1.3	0.7
45	49	1.2	0.8	1.3	1.0
50	54	1.3	0.6	1.3	0.7
55	59	1.3	0.6	1.4	1.0
Total		1.2	0.6	1.3	0.8

Table 6 Triglyceride means (mmol/l) and pathological ECG

	SD	
ECG-positive women	1.2	0.6
ECG-negative women	1.2	0.7
ECG-positive men	1.4	1.0
ECG-negative men	1.3	0.8

Serum triglycerides

The mean triglyceride value in the whole series was 1.25 mmol/l . The value for women was 1.2 mmol/l and that for men 1.3 mmol/l the difference being almost significant ($p < 0.05$) Table 5.

In women the value for triglycerides clearly increased along with age ($p < 0.001$) while in men age was not significantly related to serum triglycerides (Table 5).

Both ECG-positive and ECG-negative women had a mean triglyceride value of 1.2 mmol/l . The corresponding values in the male series were 1.4 and 1.3 which values did not differ significantly (Table 5).

The 6 ECG-positive women with a pathological Q wave had a triglyceride mean of 1.1 mmol/l which does not differ from the corresponding value of the ECG-negative women (1.2 mmol/l). The men with a pathological Q wave on the other hand had a clearly higher triglyceride mean (1.7) than the ECG-negative men (1.3) ($p < 0.001$).

Triglyceride quintiles and pathological ECG Figure 3

When the series is divided into quintiles according to the triglyceride values and the proportion of ECG positive subjects in each quintile is examined it can be seen that there are no significant differences between the groups of either sex although the triglyceride values in at least the first and the last quintile are considerably different.

The proportion of ECG-positive men is smaller in the first quintile than in the others but this difference is not statistically significant either.

ECG changes thus do not increase along with increasing triglyceride values in either sex although the variation of triglyceride values is $0.2 - 9.5 \text{ mmol/l}$ in women and $0.2 - 9.3 \text{ mmol/l}$ in men.

Summary

In the female series there is no correlation between triglycerides and pathological ECG

In the male series there is some correlation the men with a pathological Q wave in their ECG had higher serum triglyceride levels on an average than the ECG-negative men

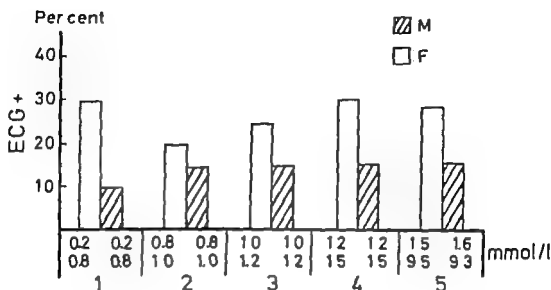
Table 5 Triglyceride means (mmol/l) in the different age groups

Age group	Women	SD	Men	SD
40-44	1.1	0.5	1.3	0.7
45-49	1.2	0.8	1.3	1.0
50-54	1.3	0.6	1.3	0.7
55-59	1.3	0.6	1.4	1.0
Total	1.2	0.6	1.3	0.8

Table 6 Triglyceride means (mmol/l) and pathological ECG

		SD
ECG-positive women	1.2	0.6
ECG-negative women	1.2	0.7
ECG-positive men	1.4	1.0
ECG-negative men	1.3	0.8

Figure 3 Triglyceride quintiles and pathological ECG



Systolic and diastolic hypertension

The mean blood pressure in the total series was 157/94 being 161/94 in the female group and 152/93 in the male group. The diastolic values do not differ from each other but women have clearly higher systolic blood pressure than men ($p < 0.001$) Table 7

In women both systolic and diastolic blood pressure increase along with age up until the age of 50 ($p < 0.001$). In men systolic blood pressure increases evenly along with age ($p < 0.01$) while diastolic blood pressure remains unchanged Table 7

The ECG-positive women have distinctly higher systolic and diastolic blood pressure than the ECG negative women ($p < 0.001$). A corresponding difference ($p < 0.001$) can also be seen in the systolic pressure of the ECG positive men but the difference in diastolic pressure is not equally clear ($p < 0.05$) Table 8

The women with a pathological Q wave in their ECG had a mean blood pressure of 160/94 which is not clearly different from the mean of the ECG-negative women. The men with a Q wave had a mean blood pressure of 157/94 the systolic pressure being clearly higher than the value for ECG-negative men ($p < 0.01$). The male groups did not differ as regards diastolic blood pressure.

Blood pressure, quintiles and pathological ECG — Figures 4 and 5

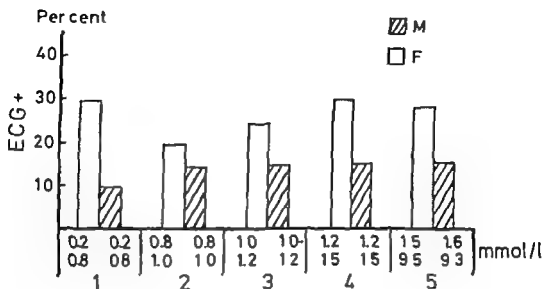
The quintiles of systolic blood pressure (figure 4) show that the proportion of pathological ECG values increases along with increasing systolic blood pressure in the female series: the difference between the first and the last quintile is almost significant ($p < 0.05$). No similar trend can be seen in the male series: only the last quintile contains more ECG-positive subjects than the other ones, but the differences between the first and the last quintile is not statistically significant.

The relationship between diastolic blood pressure and pathological ECG (figure 5) in the female group appears similar to the relationship between systolic pressure and pathological ECG: positive ECGs increase along with increasing diastolic pressure. Yet there is no significant difference between the first and the last quintile. In the male series diastolic blood pressure behaves similarly to systolic pressure: only the last quintile differs clearly from the others, but the difference is not statistically significant.

Summary

In women both the systolic blood pressure and the diastolic pressure increase up until the age of 50 and both of the pressures are higher in

Figure 3 Triglyceride quintiles and pathological ECG



Systolic and diastolic hypertension

The mean blood pressure in the total series was 157/94 being 161/94 in the female group and 152/93 in the male group. The diastolic values do not differ from each other but women have clearly higher systolic blood pressure than men ($p < 0.001$) Table 7

In women both systolic and diastolic blood pressure increase along with age up until the age of 50 ($p < 0.001$). In men systolic blood pressure increases evenly along with age ($p < 0.01$) while diastolic blood pressure remains unchanged Table 7

The ECG-positive women have distinctly higher systolic and diastolic blood pressure than the ECG-negative women ($p < 0.001$). A corresponding difference ($p < 0.001$) can also be seen in the systolic pressure of the ECG-positive men but the difference in diastolic pressure is not equally clear ($p < 0.05$) Table 8

Figure 4 Systolic blood pressure quintiles and pathological ECG

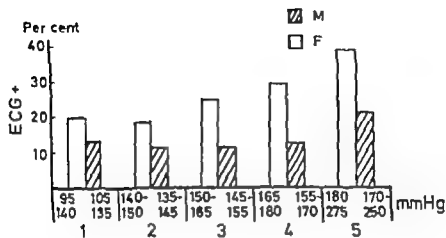
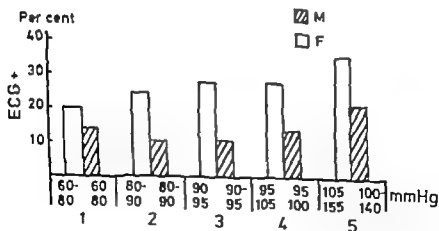


Figure 5 Diastolic blood pressure quintiles and pathological ECG



the ECG-positive subjects The ECG positive men had only the systolic pressure higher than the ECG negative men . Q pathology is only associated with elevated systolic blood pressure in men

Table 7 Blood pressure means (mmHg) in the different age groups

Age group	Women	SD	Men	SD
40 - 44	150/90	21/11	148/93	21/13
45 - 49	156/93	25/13	151/93	23/13
50 - 54	171/98	30/15	154/93	23/13
55 - 59	173/97	30/14	156/94	23/12
Total	161/94	28/14	152/93	23/13

Table 8 Blood pressure means and pathological ECG

		SD
ECG positive women	168/97	31/14
ECG-negative women	158/93	26/13
ECG-positive men	159/95	27/15
ECG-negative men	151/93	22/13

The women who had a pathological Q wave in their ECG and who have possibly suffered a myocardial infarction had a higher mean 2 hour glucose tolerance value than the ECG-negative women

Table 9 2 hour blood sugar means (mg/100 ml) in the different age groups

Age group		Women		Men	
			SD		SD
40	44	110.3	36.0	101.2	29.7
45	49	109.7	33.9	109.4	34.9
50	54	123.1	42.1	108.2	36.6
55	59	124.4	49.7	115.4	38.6
Total		116.2	40.7	108.0	35.0

Table 10 2 hour blood sugar means (mg/100 ml) and pathological ECG

		SD
ECG-positive women	119.8	40.1
ECG-negative women	114.9	40.8
ECG-positive men	109.6	34.9
ECG-negative men	107.7	34.9

Diabetes mellitus and glucose intolerance

The 2-hour mean for blood glucose was 112.1 mg/100 ml in the whole series being 116.2 in the female group and 108.0 mg/100 ml in the male group. The difference between the means of men and women is unambiguous ($p < 0.001$) Table 9.

The 2-hour mean for blood glucose increases along with age in both women ($p < 0.01$) and men ($p < 0.001$) Table 9.

The mean 2 hour blood glucose of the ECG-positive women was 119.8 mg/100 ml and that of the ECG-negative women 114.9 mg/100 ml. These values are not significantly different. The corresponding values of men did not differ significantly either. Table 10.

The ECG-positive women with a pathologic Q wave had a mean 2 hour blood glucose value of 149.2 mg/100 ml which is clearly higher than the corresponding mean of the ECG-negative women ($p < 0.001$). No parallel difference was noted in the male series.

Glucose quintiles and pathological ECG - Figure 11

When the series is divided into quintiles on the basis of the 2 hour blood glucose values it can be seen that the proportions of ECG positive subjects are the same in all quintiles of both men and women. No statistically significant differences can be seen.

Even though quintile 5 represents subjects with clearly diabetic metabolism not even this quintile included more ECG positive subjects than the others.

Summary

Depressed glucose tolerance and pathological ECG were not found to correlate in either sex group according to the quintile method.

the other ones is not significant Figure 8

In the male series the skinfold sums yield the same result as the overweight quintiles the last quintile contains fewer ECG-positive subjects than the first The differences are not significant Figure 8

An examination of the means of ECG-positive and ECG-negative subjects shows no differences in weight height or the thickness of skinfolds in either sex group Table 15

The tables 11 14 show the means for weight height and the thickness of skinfolds

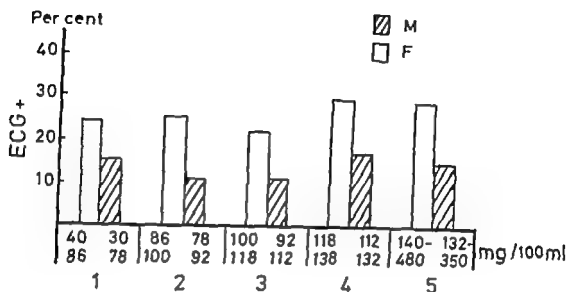
Summary

Pathological ECG thus does not clearly correlate with overweight or obesity in either sex although the women with marked overweight and obesity had somewhat more pathological ECG values

Table 11 Means

		Women	SD	Men	SD
Height	cm	156.5	5.5	168.5	6.2
Weight	kg	68.5	13.2	72.6	11.2
Triceps	mm	21.1	7.4	9.2	4.5
Subscap	mm	21.5	9.6	13.9	6.8

Figure 6 Depressed glucose tolerance and pathological ECG



Overweight and obesity

The first four of the quintiles constructed on the basis of the weight/height index (overweight) contain almost equal proportions of ECG-positive women only the last quintile includes more such subjects. The difference between the last quintile and the others ones is not however statistically significant. Figure 9

All the male quintiles include more or less equal proportions of ECG-positive subjects the last quintile has even fewer pathological ECG than the first. The differences are not significant. Figure 7

In the quintiles constructed on the basis of the skinfold sum (obesity) the proportion of ECG-positive women increases as the skin fold sum increases yet the difference between the last quintile and

Table 15 Comparison of the means of ECG-positive and ECG-negative subjects

		ECG+	ECG-	ECG+	ECG-
		women	women	men	men
Height	cm	155.8	156.7	168.2	168.1
Weight	kg	68.3	68.1	73.9	72.3
Triceps	mm	21.2	21.1	19.9	19.1
Subscap	mm	21.2	21.5	14.6	13.7

Figure 7 Quintiles constructed from the weight/height index (over weight) and pathological ECG

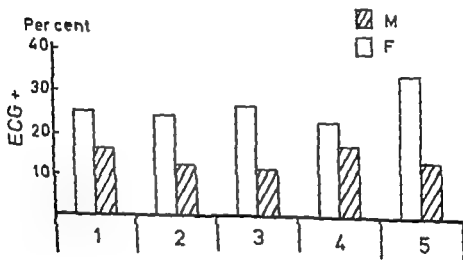


Table 12 Height means in the different age groups of women and men

	Women	SD	Men	SD
40 - 44	157.6	5.1	169.9	6.0
45 - 49	157.0	5.5	168.3	6.1
50 - 54	156.1	5.7	168.0	6.3
55 - 59	154.6	5.2	167.3	6.2

Table 13 Weight means in the different age groups of women and men

	Women	SD	Men	SD
40 - 44	67.1	13.2	73.7	11.3
45 - 49	67.9	11.9	72.9	10.9
50 - 54	70.9	14.1	72.8	11.2
55 - 59	68.6	13.3	70.6	11.3

Table 14 Skinfolds (triceps + subscapular) in the different age groups of women and men

	Women	SD	Men	SD
40 - 44	19.8 + 19.7 = 39.5	12.2	9.1 + 13.6 = 22.7	8.4
45 - 49	21.5 + 21.8 = 43.3	12.0	9.1 + 13.7 = 22.8	7.9
50 - 54	22.3 + 23.0 = 45.3	12.7	9.6 + 14.9 = 24.5	8.8
55 - 59	21.3 + 21.7 = 43.0	11.1	9.2 + 13.3 = 23.5	7.7

If we examine the division of the ECG-positive men into the different age groups we can see that the youngest age group (40-44 yr) has highly significantly fewer ECG changes ($p < 0.001$) in non-smokers than in ex-smokers or smokers. The differences level off as we move into the older age groups where the smokers, ex-smokers and non-smokers are not different except in the age group of 50-54 yr where ex-smokers have more ($p < 0.01$) ECG changes than non-smokers.

In the female series (table 17, figure 9) ECG changes were most numerous in the group of non-smokers and least numerous in the groups of smokers but no significant differences can be seen between the smoking groups.

Women have the same proportion of ECG changes in all the smoking groups of the different age groups except in the age group of 50-54 yr where non-smokers have clearly more ($p < 0.001$) ECG findings than the others.

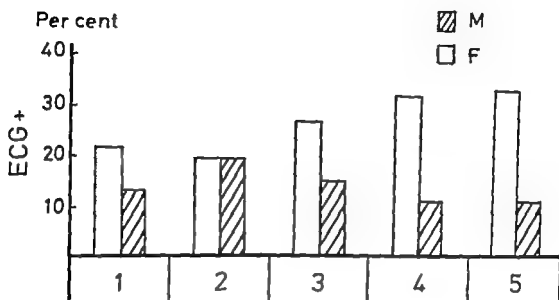
When the subjects are divided into groups on the basis of the amount of tobacco consumed (figures 11 and 12) it appears that there are equal proportions of ECG-negative and ECG-positive subjects in all male and female groups. This result has not been calculated for age groups but for the entire population investigated.

Summary

Most of the women (78%) were non-smokers while over half of the men (58.2%) were smokers.

In the female series smoking and pathological ECG did not correlate clearly while in the youngest (40-44 yr) male group non-smokers had clearly ($p < 0.001$) fewer ECG findings than smokers and ex-smokers. The older male groups displayed no correlation between smoking and pathological ECG.

Figure 8 Quintiles constructed from the skinfold sum (triceps + subscapular) (obesity) and pathological ECG



Smoking

More than half of the men were smokers (58.2 %) and an additional one quarter (24.9 %) had smoked previously. Less than one fifth of the men were non smokers (17.9 %).

In the female series an opposite situation prevailed: the majority (78.1 %) were non smokers and even ex-smokers were few in number (6.5 %).

The smokers, ex-smokers and non-smokers were divided evenly into the different age groups of both sexes. Table 16.

The ECG-positive men (table 17, figure 10) were fewest in the group of non smokers (10.4 %) and most numerous in the group of ex smokers (18.2 %). The corresponding percentage in the group of smokers was 13.3 %. These percentages are not significantly different.

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Table 16 The different smoking groups

Age group	Smokers		Ex-smokers		Non smokers	
	F	M	F	M	F	M
40 - 44	24	129	16	42	187	53
	10.6 %	57.6 %	7.1 %	18.7 %	82.3 %	23.7 %
45 - 49	34	119	12	49	148	32
	17.5 %	59.5 %	6.2 %	24.5 %	76.3 %	16.0 %
50 - 54	27	97	12	41	154	26
	14.0 %	59.2 %	6.2 %	25.0 %	79.8 %	15.8 %
55 - 59	20	92	10	55	131	23
	12.4 %	54.1 %	6.2 %	32.4 %	81.4 %	13.5 %
Total	105	437	50	187	620	134
	13.5 %	58.2 %	6.5 %	24.9 %	78.0 %	17.9 %

Table 17 Pathological ECG in the different smoking groups

Age group		Smokers		Ex smokers		Non-smokers	
		F	M	F	M	F	M
40 44		4	12	3	4	34	1
		16.7 %	9.3 %	18.8 %	9.5 %	78.2 %	1.9 %
45 49		8	19	4	8	35	6
		17.7 %	16.0 %	33.3 %	16.3 %	23.7 %	18.8 %
50 54		4	14	1	10	51	3
		14.8 %	14.4 %	8.3 %	24.4 %	38.9 %	11.5 %
55 59		8	13	3	12	51	4
		40.0 %	14.1 %	30.0 %	21.8 %	38.9 %	17.4 %
Total		22	58	11	34	171	14
		21.0 %	13.3 %	22.0 %	18.2 %	27.6 %	10.4 %

Fig. 9 Pathological ECG in the different smoking groups Women

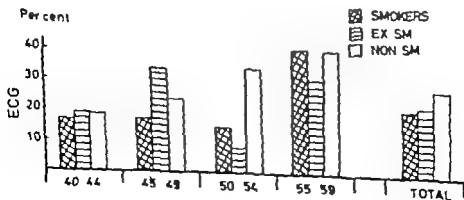


Figure 10 Pathological ECG in the different smoking groups Men

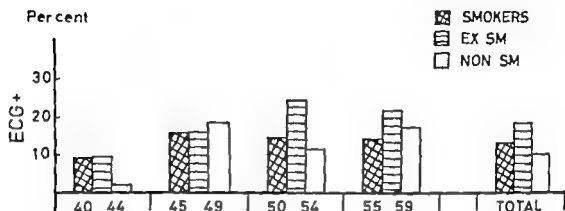


Figure 11 ECG-positive and ECG-negative subjects in the different smoking groups Women

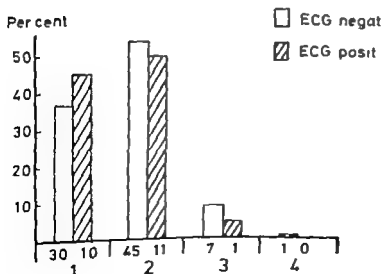
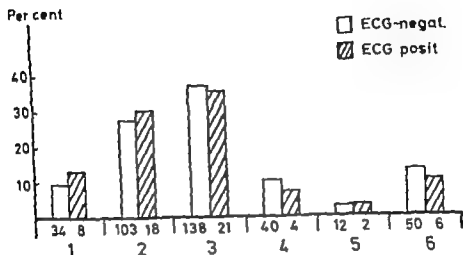


Figure 12 ECG-positive and ECG-negative subjects in the different smoking groups Men



Occupation

More than half of both men and women were engaged in heavy manual work (men 66.4 % women 56.2 %). The second biggest male group consisted of men on disability pension who accounted for as many as 16.5 % of all men. The second biggest female group (21.2 %) consisted of women doing household work (without cattle). The proportions of all the other occupational groups of both men and women were quite evenly under 10 %. Tables 18 and 19 figure 13

The men in the first four occupational groups have fairly equal proportions of ECG changes. The ECG changes were most numerous in the group of pensioners (29.6 %) and next numerous in the group of men doing household work but the small number of subjects in the

Figure 10 Pathological ECG in the different smoking groups Men

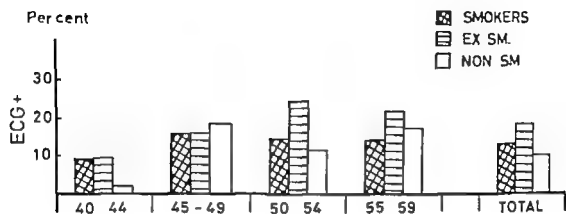


Figure 11 ECG-positive and ECG-negative subjects in the different smoking groups Women

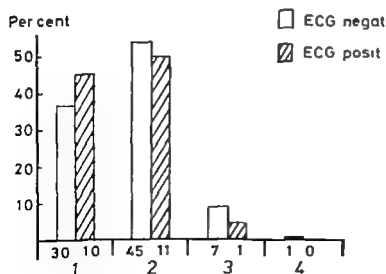
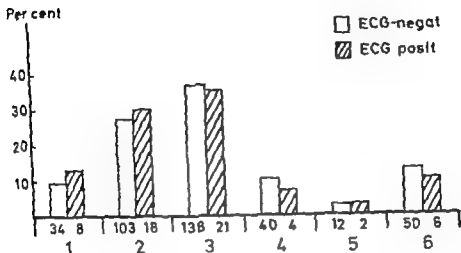


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latter group must be borne in mind Table 21 figure 17

In the female series the ECG changes were also most numerous in the group of pensioners (43.3 %) and next numerous in the group doing household work (33.8 %). The third group in frequency consisted of women doing heavy manual work of whom one fourth had pathological ECG findings (25.4 %). Group 2 (supervision teacher) included only one woman with pathological ECG out of a total of 23 women Table 20 and figure 14

Summary

The subjects on disability pension had clearly the most ECG changes particularly in the female series

In the other occupational groups men had equal proportions of ECG changes while the women doing household work any heavy manual work had the most changes

Occupational groups in the population investigated Figures 16 17
Tables 18, 19, 20, 21

- 1 = heavy manual work (farmer farmer's wife heavy household work with cattle lumbering)
- 2 = supervision stress job (teacher salesman shopkeeper doctor etc)
- 3 = office work or corresponding (clerk car transport service etc)
- 4 = standing work (shop assistant waiter/waitress nurse etc)
- 5 = household work (no cattle)
- 6 = disabled pensioner hardly any work

Table 18 19 The occupational distribution of the population investigated

Women		Occupational groups					
Age group		1	2	3	4	5	6
40	44	147	11	12	19	32	10
45	49	116	9	14	15	38	6
50	54	104	0	5	13	52	18
55	59	74	3	8	4	44	33
Total		441	23	37	51	166	67
		66.2 %	2.9 %	4.7 %	6.5 %	21.2 %	8.5 %

Men		Occupational groups					
Age group		1	2	3	4	5	6
40	44	174	15	18	5	3	10
45	49	139	15	12	4	2	27
50	54	96	11	7	6	3	40
55	59	92	12	7	2	7	48
Total		501	53	44	17	15	125
		66.4 %	7.0 %	5.8 %	2.3 %	2.0 %	16.5 %

Age group	1	2	3	4	5	6
	ECG +	ECG + %	ECG +	ECG + %	ECG +	ECG +
40 - 44	26	17.7	0	00.0	1	8.3
45 - 49	29	25.0	1	11.1	4	28.6
50 - 54	29	27.9	0	00.0	1	20.0
55 - 59	28	37.8	0	00.0	1	16.7
Total	112	25.4	1	4.4	7	18.9
					9	17.6
					51	33.8
					29	43.4

Table 21 ECG-positive subjects in the different occupational groups Men

Age group	1	2	3	4	5	6
	ECG +	ECG + %	ECG +	ECG + %	ECG +	ECG +
40 - 44	9	5.2	2	13.3	1	20.0
45 - 49	20	14.4	3	20.0	2	16.7
50 - 54	12	12.5	0	00.0	1	14.3
55 - 59	10	10.9	3	25.0	0	00.0
					0	00.0
					2	28.6
					13	27.1

Figure 13 Occupational groups in the population investigated

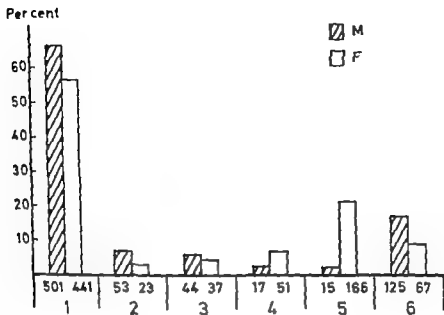
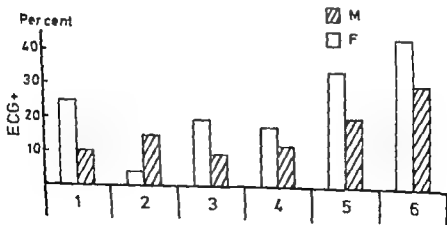


Figure 14 Pathological ECG in the different occupational groups



Correlations between the risk factors (Tree analyses)

Women

In the tree analysis of the female series the ECG variable correlated most strongly with systolic blood pressure exceeding 165 mmHg. This correlation was later accompanied by age over 50 and occupation in such a way that pensioners and women doing heavy manual work and household work were included in the correlation. Obesity was the final factor in this chain of correlations: women who are thin and have normal skinfold thickness values have more ECG changes than obese women. Figure 15

If we scrutinize the branch of lower systolic blood pressure in tree analysis we see that in addition to correlating with normal systolic blood pressure the ECG variable also correlates with occupation rendering pensioners and subjects with sedentary occupation the risk group. The last item in this chain of correlations is pathological glucose tolerance.

When the group of pensioners is excluded the ECG variable still correlates most strongly with systolic blood pressure. It is worth noticing that when systolic blood pressure is low or normal (quintiles 1, 2, 3) no other correlating factors emerge. Figure 16

The subjects with systolic blood pressure \geq 165 mmHg (the 4th and 5th quintile) have age over 50 as the next correlating factor: subjects younger than that have no additional risk factors. A hypertonic woman aged over 50 next reflects the effect of occupation: subjects with office work and standing work (shop assistant, waitress, etc.) have many ECG changes. Those doing heavy manual work and household work are clearly accompanied at the next stage by quintile 1 of the obesity

group which includes the thinnest women Quintile 5 of the obesity group for which the greatest skinfold thicknesses were recorded has already been left as a group of its own and clearly contains fewer ECG changes With regard to the occupational groups it can also be seen that group 2 (teacher shopkeeper etc) has not yet been included in the correlation

Triglycerides overweight and diastolic blood pressure appear only at the final end of the tree analysis

Summary

The tree analysis of the female series yielded the following results

- 1) Systolic blood pressure is the most important correlating factor being the only factor in women aged below 50 Low or normal systolic blood pressure did not correlate with any other factor in the analysis
- 2) Thin hypertonic women aged over 50 doing heavy manual work or household work constituted a risk group of their own Similarly hypertonic women over 50 doing office work or standing work (shop assistant waitress etc) also constitute a risk group without correlating obesity quintiles
- 3) Cholesterol and smoking were absent at all stages of the analysis
- 4) Triglycerides overweight and diastolic blood pressure appeared highly insignificant and depressed glucose tolerance entered the correlation only when it pertained to women on pension

Men

When we examine the mutual correlation of the different risk fac

tors to pathological ECG by means of the tree analysis (figure 17) we see that the strongest correlating factor is occupation. This is due to the large group of pensioners (16.5 % of men were on pension) where ECG changes were numerous. In the group of pensioners the ECG variable next correlated with smoking, and the groups of ex- and non smokers then correlated with triglycerides and age. No unambiguous result was obtained for glucose tolerance. In smoking pensioners the ECG changes correlated with high triglyceride values and high diastolic blood pressure.

The other occupational groups displayed a clear correlation: obesity (the two topmost quintiles), high systolic blood pressure (highest quintile) and high cholesterol (highest quintile) in this order. The subjects belonging to the four lowest cholesterol quintiles had the following correlation: age, heavy or stationary work, and high triglycerides.

Since it seemed expedient to examine the correlations in the group of subjects still in active work, the group of pensioners was excluded from the analysis, and another tree analysis was obtained (figure 18). An unambiguous result was obtained: a man who is thin or normal on the basis of his skinfolds and who does either heavy or stationary work is most strongly correlated with the ECG variable. No other factors affect this correlation.

Another unmistakable correlation was also established: an obese man with exceedingly high systolic blood pressure (highest quintile) and exceedingly high cholesterol (highest quintile). According to the present analysis, cholesterol is significant only in combination with high systolic blood pressure and obesity.

The obese men who were in the four lowest systolic blood pressure quintiles had overweight and triglycerides as the next correlating factors. It is worth pointing out that high cholesterol was not included

in this chain the 46 men in the last branch of this chain had no pathological ECG changes

Summary

The tree analysis of the male series yielded the following result
Two separate unambiguous risk groups emerged

- 1) a man who is thin or normal on the basis of his skinfold and who does heavy or stationary work (e.g. taxi driver)
- 2) an obese man with high systolic blood pressure and high cholesterol
Cholesterol did not emerge as an independent risk factor

The following additional observations were made
smoking was present only a feature of the group of pensioners not of the other groups
the roles of triglycerides, overweight, diastolic blood pressure and age also remained fairly insignificant
depressed glucose tolerance did not emerge as a risk factor at any stage of the analysis

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Figure 16 Tree analysis in the female series after exclusion of the pensioners

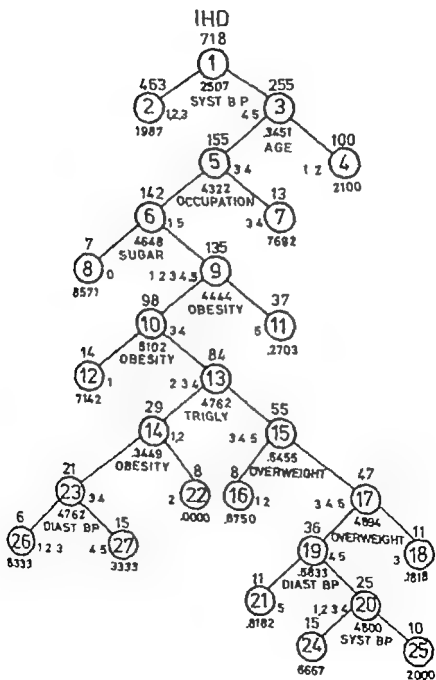


Figure 15 Tree analysis in the female series

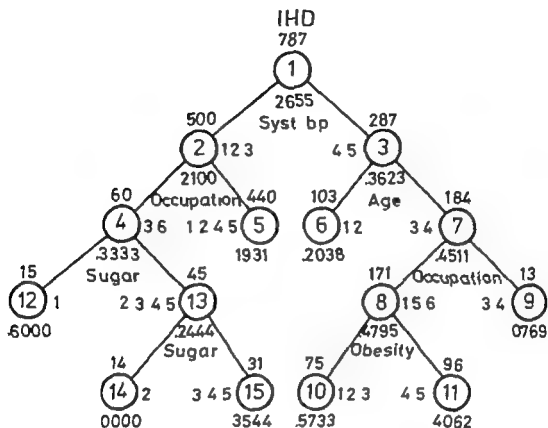
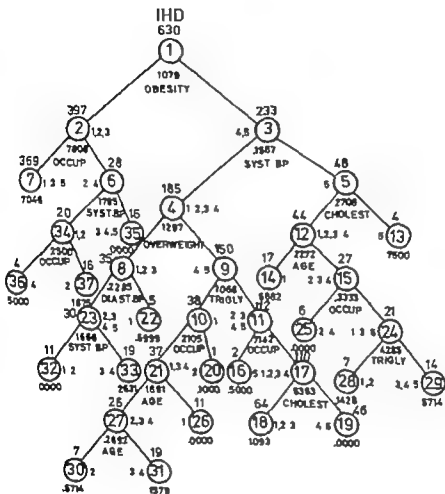
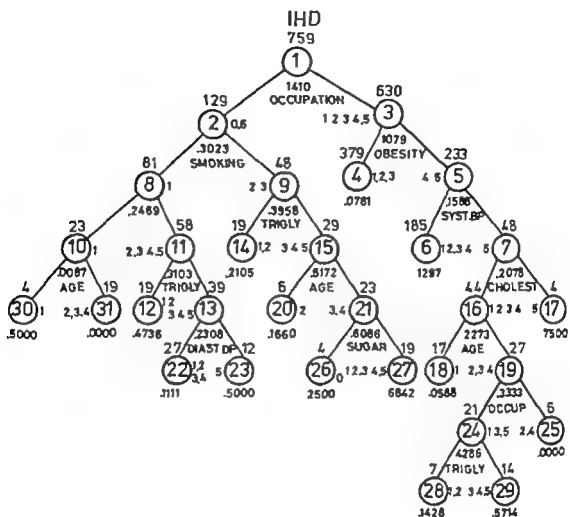


Figure 18 Tree analysis in the male series after exclusion of the pensioners





The methods applied to the different mass surveys vary. This variation also has some effect on the results making it difficult to compare the results of different mass surveys. In order to reach the best level of standardization the WHO recommendations (Rose & Blackburn 1968) were observed in the present work but even so many of the methods remain open to discussion.

Glucose tolerance test ECG

Glutodyn^R glucose solution was used in the oral glucose tolerance test which solution turned out to be well tolerated by the subjects. For practical reasons only one blood sample is taken either 1 hr or 2 hr after the administration of oral glucose in mass surveys. Since the 2 hour glucose tolerance test is considered better (Stewart and Robertson 1963 Jackson et al 1968) it was used in this series of examinations. In any case the 2 hour glucose tolerance test was well suited to the present project.

In several mass screening surveys ECG has been recorded before the intake of glucose solution directly after the fast (e.g. Pyörälä et al 1973). In some other investigations however ECG recording has been performed after the administration of glucose solution (e.g. Welborn et al 1969). The WHO instruction states: "Whenever possible meals and glucose administration should precede the ECG recording by two hours (Rose G A & Blackburn H: Cardiovascular Survey Methods. World Health Organization monograph series n o 56. Geneva 1968 page 101)". The ECG recording in this investigation was therefore performed 1.5 hr after the administration of glucose solution for practical reasons the recording could not be done after exactly 2 hours but had to be done a little earlier. Immediately after recording the ECG the blood sample for the 2 hour glucose tolerance test was taken from each subject.

DISCUSSION

General

Several methods can be used to verify IHD. Autopsy reveals sclerosis of the coronary arteries and after a certain period has elapsed since the acute phase a myocardial infarction suffered by the patient. Coronary angiography also shows the state of the coronary arteries but is inapplicable to mass surveys. Anamnestic angina pectoris is a subjective criterion which does not necessarily indicate the presence of ischemic heart disease. Electrocardiography is probably the most widely spread method for verifying IHD although it is not absolutely reliable either in the present work however ECG alone was used to verify IHD because it is the method with most documentary evidence for this use. Only severe ECG changes were accepted as signs of IHD.

Among the different mass screening surveys follow up investigations yield the best information on IHD and the factors correlating with it. A cross-section study reveals only the contemporary situation giving much more restricted information than a follow-up study. Cross sectional studies are generally accomplished within a short period and they may reveal factors which turn out as important risk factors of IHD in a longitudinal study. Still cross sectional studies always have certain limitations compared with longitudinal studies for example if a heavy smoker dies a sudden death he is not included in the cross sectional study. Conversely however the different cross sectional findings may reflect the various risk factors of IHD in different population. Taking into account the small number of the reports on IHD and risk factors in women it was considered useful to present the results of this cross section study. The intention is to continue this work as a follow up investigation.

mass screening ECG is the only standardized objective method which fulfills the condition. It is known on the other hand that the ECG changes brought about by coronary disease are not fully specific to the disease and that coronary disease does not always entail ECG changes which means that sensitivity is also restricted (Helin 1972). The T change is most sensitive to hypoxia of the cardiac muscle but at the same time least specific while the situation with the Q and QS changes is opposite.

The choice of the ECG changes to be used as criteria of IHD is in itself a problem: where should we set the limit? In the present work only relatively severe ECG changes were taken into account: of the Q changes for example only the major Q changes (Minnesota code 1 1) and of the ST/T changes only the major ST/T⁺ changes (Minnesota code 4 1 5 1 5 2) were observed.

The ST/T changes in ECG have given rise to lively discussions among investigators. Some consider the ST/T changes nonspecific either physiological or attributable to some other causes such as age (Sleeper and Orgain 1963; Rodstein et al 1966; Harriot 1967; Windsor 1968; Rotman et al 1972; Simonson 1972) while others regard the ST/T changes as being typical of ischemic heart disease mainly coronary disease (Friedberg and Zager 1961; Rose 1971; Short 1972).

According to the Framingham study (Higgins et al 1965) the subjects with a pathological isolated Q wave in their ECG had about threefold risk of dying a coronary death compared with the average: the greater the Q wave the greater the risk. Isolated negative T-waves carried a 5-fold increased risk of death and 3-fold increased risk of an incident of CHD.

Kimura and Nakayama (1972) made roughly similar observations in noting that ST depression + negative T wave were the ECG changes with the poorest prognosis. Ostrander (1970) noted that even a minor negative T wave impairs the prognosis of both women and men.

The ST/T changes in postprandial ECG were discussed in the work of Ostrander and Weinstein (1964) which covered 30 healthy men aged 22 - 66 and 23 men with coronary disease aged 39 - 86. All the postprandial ECGs of healthy subjects showed a depression of the T wave compared with the ECG recorded before glucose administration and 19 coronary patients out of 23 appeared to have the same change. Three healthy men further developed postprandial ST/T changes codable as pathological according to the Minnesota code. It should be borne in mind however that the ST/T changes noted in all the three cases were less severe than those recorded in the present study and would not have been coded as pathological in this work.

The present findings indicate that men have a roughly equal number of ECG changes at both low and high blood sugar values. For example the subjects with a 2-hour blood glucose value ranging within 30-78 mg/100 ml had as many ECG changes as the subjects with a blood glucose level of 112-132 mg/100 ml and those with a level of 132-350 mg/100 ml. The situation in the female series was similar.

It can therefore be assumed that the time of recording ECG relative to the glucose tolerance test was not responsible for false positive ST/T changes.

ECG as an indicator of IHD

In most mass screening surveys of the epidemiology of IHD the criteria of IHD have included not only certain ECG changes but also anamnestic angina pectoris syndrome and anamnestic verifiable myocardial infarction (Dawber et al 1957, Epstein et al 1965, Welborn et al 1969, Bengtsson 1973 a, Bengtsson et al 1973 b). Angina pectoris however is a subjective symptom and does not necessarily indicate the presence of ischemic heart disease. The intention of the present work was to construct an objective indicator of IHD which would be applicable to

age groups of women than in those of men. It also appears that pathological ECG findings increase evenly along with age in the female series while in the male series the prevalence of positive ECG changes remains static from the age of 45 onwards.

A majority of the ECG changes in women are ST/T changes. no pathological Q changes were noted in women aged under 50. The ST/T changes in women increase evenly along with age.

In the male series Q changes already appear in the youngest age group increasing steadily with age. Q changes account for approx one fifth of the pathological ECG changes of men. The ST/T changes of men increase sharply as we move from the youngest age group to the next whereafter their prevalence remains unchanged.

The ECG changes other than Q or ST/T changes noted in the present work are of no great significance. they accounted for 0.3 % of all changes in women and 0.8 % in men.

The present prevalence of pathological ECG findings was nearly threefold in the male series and over sixfold in the female series compared with the Framingham study (Higgins et al 1965) which covered people of the same age and employed similar criteria. Compared with the Tecumseh study (Ostrander et al 1965 a) the present prevalence of pathological ECG in the male series was nearly threefold and that in the female series about eightfold. When the Australian Busselton study (Weiborn et al 1969) is used as reference the present ECG finding in men is about threefold and that of women about tenfold.

Compared with the studies conducted by Pyörälä et al (1973) in different parts of Finland the present prevalence of ECG findings is about twofold in the male series and about 2.5-fold in the female series. Pyörälä's work showed the frequency of ST/T changes in women to be over twofold compared with those in men. An interesting detail in Pyörälä's findings might be pointed out here: numerous ECG findings were found at

78
According to the investigations of Short and Stowers (1972) a ST depression of only 0.5 mm combined with a negative T wave is a sign of clear ischemic heart disease. They call such a change a major ST/T change while depressions of 0.25 - 0.5 mm are termed minor changes which are also pathological and are usually due to either coronary disease or left ventricular hypertrophy.

Rose (1971) also emphasizes the minor ST/T changes of ECG on the basis of his studies. He draws attention to the curious fact that so many coronary deaths and myocardial infarctions seem to be completely unexpected catastrophes. He postulates that minor symptoms and minor ECG changes may occur in the years before these unexpected illnesses. In a previous (1968) paper Rose pointed out that the prevalence of IHD is underestimated in mass screening surveys, i.e. IHD is more common in reality than is shown by the prevalence figures.

Raftery et al. (1971) found that coronary patients may have a pathological ECG on certain occasions while at some other time the ECG may be normal, which means that the ECG changes indicative of coronary disease may vary. Accordingly ST/T changes once noted bear a great significance as indicators of ischemic heart disease.

Many authors thus emphasize the role of even minor ST/T changes (0.25 - 0.5 mm depression of ST) as indicators of ischemic heart disease. The ST/T changes interpreted as pathological in the present work were more serious.

ECG finding

Attention is drawn to the great number of ECG changes although the criteria used in the interpretation were fairly strict. Another finding which invites attention is the greater number of ECG changes in all the

This matter was mentioned by Punsar & Karvonen (1973) in discussing the relations between prevalence and incidence. According to them two populations with the same prevalence of ECG changes need not have the same CHD incidence or mortality because the symptomatic stage of CHD may be different in different populations. Hence in theory a low prevalence of ECG changes may be due to a short duration of CHD and a high prevalence of ECG changes may reflect a low mortality from the disease.

Hellä (1976) noted that atherosclerosis of the coronary arteries is noticeably more common among men than women. However it appears that the clinical symptoms and signs resulting from coronary artery stenosis occur in women at a lower degree of narrowing than in men.

When discussing the frequency of ECG changes we must also bear in mind the great number of pensioners in the series investigated. As many as 16.5 % of the men (125 men) were on pension, of whom nearly a third (29.6 %) had ECG changes. 8.5 % of the women (67 women) were on pension and almost examined represent the working age population, the retirement on pension must have been due to some disease. Taking into account the numerous ECG changes in the group of pensioners one can postulate heart disease as a common cause for retirement. Anyway the high frequency of ECG findings among the pensioners increases the prevalence of pathological ECG in the whole series.

Serum cholesterol

Cholesterol has been considered perhaps the most important risk factor of IHD both in men and in women aged below 50 (Giannini 1970, Kannel 1971, Stamler 1973). In women over 50 the significance of cholesterol as a risk factor of IHD is less clear (Kannel and Castelli 1972).

It has also been maintained that determination of the serum cholesterol level is the best simple means of estimating the risk of atherosclerotic

Merijärvi which is one of the neighbouring municipalities of Haapavesi where the present investigations were carried out. At Merijärvi the prevalence of female T changes (Minnesota codes 5.1 - 5.3) was over 30 % the corresponding figure for men being nearly 15 % which values clearly exceed the prevalence of T changes noted in the present work. It must be noted however that the ECG coding used by Pyörälä et al included changes of less severe degree than the coding in this investigation.

According to the East West study (Punsar & Karvonen 1973) the prevalence of ECG changes in men was 5.7 % in the west and 6.7 % in the east at the beginning of the survey in 1959. Hence the present prevalence of male ECG changes was about twofold compared with the corresponding finding of the East West study. It should be borne in mind however that the criteria of ECG coding differed somewhat in these two works.

Why were there so many ECG changes? It is a well known fact that even experienced clinicians differ in their interpretation of ECG (Epstein et al 1961) but errors in interpretation can probably not explain the great number of ECG findings. Nor does the effect of possible glucose intake appear an adequate explanation.

It is known on the other hand that ischemic heart disease is exceptionally common in Finland. Hence the pre stage of IHD might also be common. Some of the subjects suffering from such latent IHD develop clinical symptomatic ischemic heart disease while others do not develop it. The great number of ECG findings might suggest that a large proportion of the population suffers from subclinical IHD.

ECG findings were numerous in both sexes but were nearly twice as frequent in women as in men. The ECG changes in women were however clearly milder which might be taken to indicate that the clinical manifestations of IHD are milder in middle aged women than men.

blood pressure quintile (170 mmHg) and the two highest obesity quintiles when however the systolic blood pressure was below 170 mmHg high cholesterol together with obesity and overweight were not connected with pathological ECG in men

Serum triglycerides

The significance of triglycerides as a risk factor of IHD has been emphasized in several investigations over the last few years (e.g. Carlson & Böttiger 1972 Kannel & Castelli 1972)

The present studies showed that the women with exceedingly low triglyceride values (0.2 - 0.8 mmol/l) had the same frequency of pathological ECGs as the women with high triglycerides (1.5 - 9.5 mmol/l)

Men on the other hand had fewer ECG changes in the lowest triglyceride quintile than in the other four quintiles but the difference was not significant

In the female series the triglyceride values clearly increased along with age as it was earlier noted that the ECG changes increased along with age. It could have been expected therefore that the number of pathological ECGs would have increased parallel with the rising triglyceride values but this was not the case

The ECG-positive and the ECG-negative women did not differ in the mean triglyceride values. The 22 men who had a Q wave (Minnesota code 11) signifying a necrotic change in their ECG however had a higher triglyceride mean (1.7 mmol/l) than the ECG-negative men (1.3)

Even in the tree analysis the role of triglycerides as a risk factor remained quite invisible in both the male and the female series. In both sex groups the triglycerides only emerged at a late stage and no clear correlation appeared

disease particularly coronary disease (Stamler 1973)

In the present study cholesterol increased along with age parallel with the increase of the prevalence of pathological ECG. It was noted however that the men with a cholesterol level of 155 - 232 mg/100 ml had the same frequency of pathological ECG findings as the men with a cholesterol level ranging from 306 to 456 mg/100 ml. The situation in the female series was more or less similar no significant differences were noted in the frequency of pathological ECG whether the cholesterol level was low (89 - 224 mg/100 ml) or high (310 - 441 mg/100 ml). Nor did the ECG-positive and the ECG-negative subjects differ as regards mean cholesterol in either sex group. Not even the subjects with a pathological Q change in their ECG had a cholesterol mean different from the corresponding mean of the ECG-negative subjects.

The present work thus gave no evidence of a correlation between serum cholesterol and IHD when cholesterol was viewed as a single risk factor. Werkö (1976) analyzed critically the findings of three comprehensive epidemiological studies (the Framingham Study, the National Pooling Project and the Stockholm Prospective Study) and concluded that none of the risk factors is very important alone. According to him the worst combination is that between smoking and hypertension or high cholesterol or both. The study by Wilhelmsen et al (1973) similarly revealed hypertension, high cholesterol and cigarette smoking to be a combination indicative of a high risk of myocardial infarction.

In the present combination analysis (tree analysis) cholesterol was absent from all the branches of the tree analysis of women which means that none of the present methods showed cholesterol to be a risk factor of IHD in the female group. In the male series cholesterol was clearly connected with the ECG variable together with high systolic blood pressure and obesity. This correlation involved the highest cholesterol quintile (cholesterol 306 mg/100 ml).

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Summarizing we can say that none of the present methods showed either triglycerides or cholesterol to be a risk factor of IHD in the female series. In the male series too the correlation between triglycerides and IHD remained uncertain although the finding suggested that a high triglyceride level might correlate with severe IHD.

Systolic and diastolic hypertension

Many of the earlier studies have shown conclusively that both systolic and diastolic hypertension is a risk factor of IHD in both sexes (e.g. Stamler 1973, Kannel 1974). The importance of early and effective treatment of hypertension is emphasized (Veterans Administration Co-operation Study Group on Antihypertensive Agents 1970) although it has not been proved that treatment of hypertension as such would definitely reduce coronary events.

The present findings showed that the systolic and diastolic blood pressure of women and the systolic blood pressure of men increased along with age (40-59 yr). The diastolic pressure of men remained unchanged despite increasing age.

It was further noted that the ECG-positive women and men had higher systolic and diastolic blood pressure than the ECG-negative subjects. The difference in the diastolic pressure of men was not great however though it was statistically significant ($p < 0.05$).

When the relationship between hypertension and IHD was examined in the different quintiles the highest blood pressure quintile was found to include more ECG-positive men and women than the lowest quintile. A significant ($p < 0.05$) difference was only noted between the systolic pressures of women. The proportion of ECG-negative women increased evenly along with elevation of both systolic and diastolic blood pressure. No similar trend was noted in the male series for only the highest blood

pressure quintile (170-250/100-140) was found to contain more ECG-findings than the other quintiles

Women were found to have a clearly higher ($p < 0.001$) mean systolic blood pressure than men while the mean diastolic pressure was of the same order in the two groups. Since women also had more ECG changes than men it can be surmised that the ECG findings of women are partly explained by the higher blood pressure. The significance of blood pressure is suggested by the fact that women had more ECG changes than men at the same blood pressure value (fig. 4-5).

It should also be borne in mind however that the lowest blood pressure quintile included relatively many ECG-positive subjects in both sex groups. The female group with low blood pressure (95-140 mmHg) included a major proportion of ECG-positive subjects, or 20.8% (the proportion of ECG-positive women in the total series was 26.5%). Correspondingly the lowest diastolic pressure quintile (60-80 mmHg) included 20.1% ECG-positive women.

The situation in the male series was more or less similar when systolic blood pressure was low (105-135 mmHg) ECG-positive subjects accounted for 13.1% (the proportion of ECG-positive men in the total male series was 14.3%) and when diastolic pressure was low (60-80 mmHg) the percentage of ECG-positive men was also high, 14.4%.

In the tree analysis the role of systolic blood pressure appeared very clearly in both sex groups. In the total female series pathological ECG was accompanied by high systolic blood pressure, age over 50, heavy work or retirement and the lowest obesity quintiles. In the group of women aged under 50 systolic blood pressure was the only risk factor and if the subject belonged to one of the lowest three blood pressure quintiles (systolic blood pressure ≤ 165 mmHg) no other risk factors emerged. In the group of hypertonic women aged over 50 ECG change

correlated with office and standing work no further explanation was provided by the tree analysis

In men high systolic blood pressure was closely associated with the ECG variable together with obesity and high cholesterol. In the group of retired men however high systolic blood pressure did not explain the incidence of pathological ECG

The role of diastolic blood pressure was small in the tree analysis of both sex groups it appeared only at the last few branches and provides no ground for conclusions

The present findings suggest that systolic blood pressure is the most important risk factor of IHD in women being the only risk factor in women under 50 years old. IHD and systolic blood pressure also clearly correlate in the male group and systolic hypertension is further accompanied by obesity and high cholesterol. No firm conclusions can be drawn concerning diastolic blood pressure in either sex group

Diabetes mellitus and glucose intolerance

A clear correlation between depressed glucose tolerance and IHD has been noted in both women and men by several investigators (e.g. Kannel et al 1967 a, Stamler and Epstein 1972). According to the Framingham report (Garcia et al 1970) CHD was particularly frequent in women taking insulin but it has also been found to correlate with milder forms of diabetes (Weaver et al 1970). Yet many authors consider diabetes mellitus and depressed glucose tolerance a secondary risk factor of CHD (Simborg 1970, Stamler 1973).

The present study revealed that the mean 2 hour glucose value was higher ($p < 0.001$) in women than in men and that this mean clearly ($p < 0.001$) increased along with age in both sexes. Since it was earlier demonstrated (fig. 1) that the prevalence of pathological ECG

increased along with increasing age it could be expected that it would also increase along with rising 2 hour glucose value but this was not the case. All the glucose quintiles contained equal numbers of ECG-positive subjects in both sex groups. Nor did the 2 hour glucose means differ in the sex groups.

If we further examine the glucose quintiles (fig. 6) we can see that the proportion of ECG changes was essentially the same regardless of whether the 2 hour blood glucose value was low (quintile 1), moderate (quintile 3) or high (quintile 5). In other words, the low or high level of blood glucose has no effect on the ECG finding.

The women with a pathological Q wave in their ECG (Minnesota code 11) had a clearly higher 2 hour mean of blood glucose than the ECG-negative women. But as there were only 6 such women, no conclusions can be drawn.

In the tree analysis, depressed glucose tolerance only appeared in the group of women on pension, whereas the male group appeared to be unaffected by it.

Overweight and obesity

Contradictory views have been presented concerning the correlation between overweight and obesity on the one hand and IHD on the other. Obesity has been assumed to correlate with angina pectoris and sudden deaths due to arrhythmia, but its relation to coronary disease has been unclear (e.g. Kannel et al. 1967; Heyden et al. 1971).

The women taking part in the present investigation were relatively fat and the men relatively thin (table 11). The height, weight and skinfold measurements of the ECG-positive and the ECG-negative subjects did not differ in either sex group.

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blood pressure quintile. In the male series the ECG variable was associated with the thin subjects rather than the obese ones.

Thinness as a risk factor was thus associated in both sexes with occupation while obesity together with high systolic blood pressure and high cholesterol was a risk factor of men. The obesity of women did not correlate clearly with the ECG variable.

Smoking

The proportion of smokers in the male series was 58.2%. In the East-West study (Key et al 1967) 68.5% of the eastern men and 57.2% of the western men smoked cigarettes. When the ex-smokers are included altogether 83.1% of the present male subjects are smokers (58.2% + 24.9%). The corresponding value in the Norwegian series investigated by Zeiner-Henriksen (1976) was of the same order 78%. The proportion of smokers is similar in all age groups while ex-smokers are more numerous in the oldest age group (54-59 yr) than in the others and non-smokers less numerous in that group than in the others.

Only 12.4% of the women were smokers and 6.5% ex-smokers totalling 18.9%. The corresponding value in Zeiner-Henriksen's series (1976) was 25%. The proportions of smokers were similar in the different age groups of women.

The correlation between smoking and IHD was clearly visible in the youngest group of men (40-44 yr) where the non-smokers included only 1.9% ECG-positive men. The proportion of ECG-positive smokers and ex-smokers was over 9% (9.3% and 9.5%). This difference is highly significant. It should further be pointed out that there were 53 non-smoking men in this age group and only one of them had pathological ECG. In the next age group (45-49 yr) ECG changes were equally numerous in all the different smoking groups but in the group aged 50-54 ex-smokers had

The women who were considerably obese and had marked overweight had more ECG changes than the others but the difference was not statistically significant

The ECG changes of men were equally numerous in all the quintiles regardless of the structural type

According to several investigations (e.g. Stamler et al 1966 Keys et al 1972) obese subjects are more inclined to hypertonia, diabetes and hyperlipidemia than thin ones and hence obesity correlating with several other risk factors constitutes a marked risk. The observations of both Stamler (1967) and Kannel et al (1971) showed the progressive and apparently synergistic effect of the presence of two, three or four risk factors.

Both the thin and the fat subjects of the obesity group play a conspicuous role in the present multivariate analysis. In the female series one risk group consisted of thin hypertonic women aged over 50 doing heavy work or household work. In the male series the obesity correlated most strongly with the ECG variable after exclusion of the subjects on pension. In the male series obesity was generally associated with pathological ECG together with high systolic blood pressure and high cholesterol. It was significant however that men who were thin or normal on the basis of their skinfolds and who were doing heavy or stationary work made up a risk group of their own. No other risk factors were combined with this correlation in the analysis.

The overweight variable only pertains to the working subjects when the subjects on pension were included in the tree analysis. This variable did not appear in either sex group. After the exclusion of retired subjects too the contribution of overweight as a correlating factor seems quite small. In the female series the overweight variable was associated with high triglycerides and the highest diastolic

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the series examined considerably less than men

In the present work the correlation between IHD and smoking was clearly apparent in the youngest age group of men and in the group of men on pension which means that the present finding agrees with the results of the previous studies of incidence and mortality. The present findings yielded no evidence of any correlation between IHD and smoking in women.

Occupation

In both sexes the largest occupational group consisted of subjects doing heavy manual work. They accounted for 56.2 % of women and 66.2 % of men. The women in this group were generally wives of small farmers whose household chores were supplemented by heavy work in cattle raising. The men did normal agricultural work and additional lumbering in the winter for further earnings.

The second largest group of women consisted of those doing only household work which may often be quite heavy. They accounted for 21.2 % of the women. The third largest female group was that of pensioners 8.5 % and next came the women doing "standing" work i.e. shop assistants, waitresses, nurses, etc. who accounted for 6.5 % 4.7 % of the women were doing office work and the smallest group consisted of 23 teachers, shopkeepers, etc. accounting for 2.9 %.

The second largest group of men was that of pensioners (16.5 % or 125 subjects) following the subjects with heavy manual work. Thus every sixth of the 761 men aged 40-59 was on pension for some disease or reason. The other male groups were clearly smaller: group 2 (teacher, salesman, shopkeeper, etc.) 53 subjects or 7 %; group 3 (office work, car transport service, etc.) 44 subjects or 5.8 %. The smallest male groups were group 4 (waiter, shop assistant and other standing work) consisting of 17 subjects or 2.3 % of all men and group 5 (household

clearly more ECG changes than the others. This can be a coincidence or it may mean that smoking had been given up either spontaneously or at the doctor's advice when the IHD symptoms appeared. Taking into account all the age groups, ECG changes were most numerous among ex-smokers and least numerous among non-smokers (18.2 % and 10.4 %) but the difference was not quite significant.

The ECG changes of women were most numerous in the group of non-smokers and least numerous in the group of smokers but no significant differences appeared (27.6 % and 21.0 %). In the group aged 50 - 54 non-smokers had highly significantly more ECG changes than smokers or ex-smokers. It must be pointed out, however, that women were generally few in the groups of smokers and ex-smokers and that this age group for example included 27 smokers and 12 ex-smokers.

The smokers were further divided into groups on the basis of the quality of smoking and the number of cigarettes consumed. There were no cigar smokers and all the pipe smokers were men. ECG-positive and ECG-negative subjects were equally numerous in all the smoking groups of both women and men. The age groups were not specified in this analysis but the whole series was treated as one unit.

The tree analysis did not reveal smoking at all in the female series and in the male series it appeared only in the group of pensioners where it seemed to correlate strongly with the ECG variable.

Having reviewed the findings of three comprehensive epidemiological studies, Werkö (1976) concludes that smoking together with high blood pressure is the most important risk factor of IHD. The IHD mortality of men has also been clearly connected with smoking in most studies (e.g. Punsar and Pyörälä 1970, Zeiner-Henriksen 1976). No equally clear evidence is so far available for the relation between IHD and smoking in women. The reason for this may be that women smoke or have smoked in

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The smoker were further divided into groups on the basis of the quality of smoking and the number of cigarettes consumed. There were 100 cigar smokers and all the pipe smokers were men. ECG-positive and ECG-negative subjects were equally numerous in all the smoking groups of both women and men. The age groups were not specified in this analysis but the whole series was treated as one unit.

The tree analysis did not reveal smoking at all in the female series and in the male series it appeared only in the group of pensioners where it seemed to correlate strongly with the ECG variable

Having reviewed the findings of three comprehensive epidemiological studies, Werko (1976) concludes that smoking together with high blood pressure is the most important risk factor of IHD. The IHD mortality of men has also been clearly connected with smoking in most studies (e.g. Fomsar and Bjorala 1970, Leiner-Henriksen 1976). No equally clear evidence is so far available for the relation between IHD and smoking in women. The reason for this may be that women smoke or have smoked in

work (e.g. car transport service) constituted a risk group without any other correlating factors. The thin women doing heavy manual work or household work also made up a risk group of their own but this chain of correlations was supplemented by high systolic blood pressure (165 mmHg) and age over 50. Also age over 50 and hypertension were linked with occupational groups 3 and 4 (office work standing work e.g. shop assistants and waitresses) in the female series. Attention is further invited to the fact that the female group 2 (teacher stress job) has been left out of the correlations entirely this group however included only 23 subjects as it was pointed out above.

Several earlier investigations have shown that CHD and inactive occupation are correlated (e.g. Morris et al 1953 Wilhelmsen and Tibblin 1971). Conversely a correlation has also been established between heavy work and CHD (Karvonen et al 1972). Poor social conditions (low social class low income level low educational level social alcohol problems) have also been found to be associated with an increased frequency of myocardial infarctions and sudden deaths (Merkb 1976). The present findings suggest that heavy work and IHD are correlated but it must be taken into account that more than half of the present subjects of both sexes did heavy manual work. It should further be emphasized that the number of pensioners in the series is great about every 6th man and about every 12th woman were on pension because of some disease or other reason and the groups of pensioners of both sexes had the most ECG changes.

work) : comprising 15 subjects or 20 %

ECG changes were most numerous in the groups of pensioners of both sexes 43.4 % of the female pensioners (29) and 29.5 % of the male pensioners (37) had pathological ECG findings

The concentration of ECG changes in the group of pensioners seems natural for it can be assumed that a large proportion of these subjects had retired because of cardiovascular defects. Also the high proportion of pensioners in the total series and the numerous ECG findings made among them explain the great number of ECG changes in the whole series

The second highest frequency of ECG changes was recorded for the groups of men and women doing household work. In the female series however the group doing heavy manual work (group 1) had almost equally many ECG findings. It can be postulated that the men in the household group have taken up lighter work of this kind being unable to do heavier work because of IHD or other symptoms and being not yet granted a pension. It must be remembered however that this group includes only 15 men.

The other occupational groups of men have roughly equal proportions of ECG changes. One further point of interest group 2 of women (teacher shoemaker stress job) contains only the pathological ECG change this group does not however include more than 23 women. Moreover most (20) of the women in group 2 are under 50 years old.

The role of occupation was clearly visible in the tree analyses. In both sexes the ECG variable correlated strongly with the group pensioners although these subjects had not retired particularly because of IHD. Anyway ECG changes were most numerous in the group of pensioners among both men and women.

When the pensioners were excluded from the analysis the role of occupation appeared clearly thin or normal men doing heavy or stationary

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CONCLUSIONS

The results obtained by using the present methods give rise to the following conclusions

- The prevalence of IHD was very great in both men and women when accepted ECG changes were used as the criteria of IHD. Women had more IHD than men in all age groups but the IHD of men seemed to be a more severe kind on the basis of the Q changes
- Systolic hypertension was the most important risk factor of IHD in the female series being the only risk factors in the group of women under 50. In the male series systolic hypertension was not an independent risk factor but it correlated clearly with IHD together with obesity and high cholesterol
- Smoking appeared clearly as an independent risk factor in the youngest (40 - 44 yr) group of men. No other age groups of men had smoking as a risk factor but in the group of pensioners smoking re appeared as a correlating factor of IHD. In the female series smoking and IHD appeared to have no correlation
- Cholesterol did not emerge as a risk factor in the female series. In the male series it was not an independent risk factor either but was associated with IHD together with obesity and high systolic blood pressure
- Each sex had a risk group for occupation and IHD 1) thin women over 50 doing heavy manual work or household work with systolic blood pressure ≥ 165 mmHg 2) men who are thin or normal on the basis of their skinfolds and who do either heavy or stationary work

SUMMARY

In the spring 1971 a mass screening survey was carried out in the municipality of Haapavesi (7500 inhabitants) in Northern Finland. All the women and men aged 40-59 years living within the municipality were invited to participate. The examinations were attended by 761 men and 793 women making the percentage of participation 90.9 %.

The correlation between ischemic heart disease (IHD) and the following risk factors was investigated: serum cholesterol, serum triglycerides, systolic and diastolic hypertension, depressed glucose tolerance, obesity and overweight as well as smoking and occupation.

Electrocardiography alone was used as the indicator of IHD when the ECG changes were of relatively severe degree. The subjects whose ECG was found to include one of the following changes according to the Minnesota code were classified as ECG-positive (pathological ECG):

- 1) Minnesota code 1.1 ("major Q")
- 2) Minnesota code 4.1, 5.1, 5.2 (major ST/T depression)
- 3) Minnesota code 8.1 (complete A-V block)
- 7.1 (complete left bundle branch block) and 8.3 (atrial fibrillation)

The ECG-positive women numbered 210, accounting for 26.5 % of the total female series. There were 202 ST/T changes (96.2 %), 6 Q changes (2.9 %) and 2 other changes (1.0 %).

The ECG-positive men numbered 109 (14.3 %) of whom 22 (20.2 %) had Q changes, 81 (74.3 %) ST/T changes and 6 (5.5 %) other changes.

The women had more ECG changes than men in all the age groups, but the ECG changes in men were more severe than those of women.

In the female series the frequency of pathological ECG changes clearly increased along with increasing age, while in the male series it increased from the youngest (40-44 yr) age group to the next (45-59 yr). From the age of 45 onwards the prevalence of pathological ECG remained unchanged in the male group.

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- Systolic hypertension was the most important risk factor of IHD in the female series being the only risk factors in the group of women under 50. In the male series systolic hypertension was not an independent risk factor but it correlated clearly with IHD together with obesity and high cholesterol

Smoking appeared clearly as an independent risk factor in the youngest (40-44 yr) group of men. No other age groups of men had smoking as a risk factor but in the group of pensioners smoking re-appeared as a correlating factor of IHD. In the female series smoking and IHD appeared to have no correlation

Cholesterol did not emerge as a risk factor in the female series. In the male series it was not an independent risk factor either but was associated with IHD together with obesity and high systolic blood pressure

- Each sex had a risk group for occupation and IHD. 1) thin women over 50 doing heavy manual work or household work with systolic blood pressure ≥ 165 mmHg. 2) men who are thin or normal on the basis of their skinfolds and who do either heavy or stationary work.

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The correlations between the different risk factors and IHD were examined by dividing the men and women into quintiles on the basis of the numerical value of each risk factor and by recording the proportion of pathological ECGs in each quintile. Moreover, the mutual correlations between IHD and the different risk factors were analyzed by means of tree analysis.

The following results were obtained for the correlations between IHD and the different risk factors:

1 Cholesterol

In the female series cholesterol and IHD did not correlate at all.

In the male series cholesterol did not emerge as an independent risk factor, either, but was associated with IHD together with systolic hypertension and obesity.

2 Triglycerides

The women showed no clear correlation between triglycerides and IHD. The men had a correlation when IHD was very severe.

3 Hypertension

Systolic hypertension was clearly the most important risk factor of IHD in the female series, being the only risk factor in the women aged under 50. In the male series, too, systolic hypertension and IHD clearly correlated, though the correlation was not equally distinct to that in the female series. In the male series, a chain of correlations consisted of systolic hypertension, obesity and high cholesterol.

The significance of diastolic blood pressure was overshadowed by systolic blood pressure in each sex group.

4 Depressed glucose tolerance

Depressed glucose tolerance did not correlate clearly with IHD in either sex group

5 Overweight and obesity

Overweight and IHD did not correlate clearly in either sex group
The obesity variable and IHD however exhibited some correlations

1) thin hypertonic women aged over 50 doing heavy manual work or household work 2) thin or normal men doing heavy or stationary work and 3) obese men with high systolic blood pressure (≥ 170 mmHg) and high cholesterol (≥ 306 mg/100 ml)

6 Smoking

Smoking and IHD did not seem to correlate in the female series
In the male series smoking emerged as an independent risk factor in the group of the youngest men (40-44 yr) and another time though not so markedly in the group of pensioners

7 Occupation

The correlation between occupation and IHD appeared in both sex groups see point 5 It should still be emphasized that thin men doing heavy or stationary work constituted a risk group without any other correlating factors

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Table 23 Differences of the mean age in the different quintiles - Women

	quintiles	statist difference
Cholesterol	2 4	$p < 0.05$
	2 5	$p < 0.05$
Triglyc	1 2	$p < 0.05$
	1 3	$p < 0.05$
	1 5	$p < 0.01$
	4 5	$p < 0.05$
Syst BP	1 3	$p < 0.05$
	1 4	$p < 0.001$
	1 5	$p < 0.001$
	2 4	$p < 0.01$
	2 5	$p < 0.001$
	3 5	$p < 0.001$
	4 5	$p < 0.05$
Diast BP	1 4	$p < 0.01$
	1 5	$p < 0.001$
	2 3	$p < 0.05$
	2 4	$p < 0.001$
	2 5	$p < 0.001$
Blood glucose	1 5	$p < 0.01$
	2 5	$p < 0.01$
	4 5	$p < 0.01$
Weight/height	1 5	$p < 0.05$
	2 5	$p < 0.05$
	3 5	$p < 0.05$
Skinfolds	1 4	$p < 0.05$
	2 4	$p < 0.05$

No other differences between quintiles were found

APPENDIX

Table 22 The mean age in the different quintile : Women

Quintile	1	2	3	4	5
Cholesterol	49.3	48.8	50.9	51.3	51.2
Triglyc	48.5	50.7	51.4	49.9	52.0
Syst. BP	46.8	47.8	49.6	51.5	53.7
Diast. BP	48.2	48.0	51.1	51.9	52.8
Blood glucose	48.6	48.3	50.8	49.7	52.7
Weight/height	49.8	49.1	49.4	51.5	52.1
Skinfolds	48.9	48.9	50.7	52.4	50.6

Table 24 The mean age in the different quintiles Men

Quintile	1	2	3	4	5
Cholesterol	50.7	48.8	50.7	51.0	48.9
Triglyc	49.0	48.4	53.3	49.5	51.1
Syst BP	49.3	50.1	49.6	50.9	50.6
Diast BP	49.9	48.4	52.1	49.9	51.1
Blood glucose	50.6	47.6	49.5	49.6	53.1
Weight/height	49.3	50.6	49.8	49.5	51.1
Skinfolds	48.1	50.6	49.7	51.0	50.8

Table 25 Differences of the mean age in the different quintiles Men

	quintiles	statist difference
Triglyc	1 - 3	$p < 0.05$
	2 - 3	$p < 0.01$
	3 - 4	$p < 0.05$
Diast BP	2 - 3	$p = 0.05$
Blood gluc	2 - 5	$p < 0.01$
	3 - 5	$p < 0.05$
	4 - 5	$p < 0.05$

No other differences between quintiles were found

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Metabolism of plasma lipoproteins

By Ole Faergeman

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- 3) Faergeman O Sata T Kase J.P & Havel R.J Metabolism of apoprotein B of plasma very low density lipoproteins in the rat Journal of Clinical Investigation 56 1396-1403 1975

Abbreviations used in this review

apo	ipoprotein
HDL	high density lipoproteins
LCAT	lecithin cholesterol acyltransferase
LDL	low density lipoproteins
VLDL	very low density lipoproteins

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Introduction

The best understood and perhaps most important function of the plasma lipoproteins is transport of triglycerides in chylomicrons and very low density lipoproteins (VLDL) from the small intestine or the liver to adipose tissues or muscle. The latter tissues contain in their capillary wall an enzyme lipoprotein lipase that hydrolyzes the triglyceride of chylomicrons and VLDL without removing the whole lipoproteins from the plasma. The resulting progressively smaller remnant lipoproteins are either removed from plasma in the liver or converted to low density lipoproteins (LDL) which in turn may be catabolized in non-hepatic tissues. High density lipoproteins (HDL) contain little triglyceride but they do carry proteins serving as cofactors for lipoprotein lipase. HDL are also closely associated with lecithin:cholesterol acyltransferase (LCAT), cholesterol esterifying enzyme of importance in plasma cholesterol transport.

The amount of available information about the plasma lipoprotein system outlined above (Fig. 1) is so large that no recent reviews have encompassed it. The present one will be no exception. Prefaced by brief discussion of lipoprotein structure and synthesis, it will deal mainly with information and ideas about the metabolism of the triglyceride-rich lipoproteins. Pertinent to the reviews will be reviewed frequently.

Structure and composition of lipoproteins

Triglyceride and cholesteryl esters are hydrophobic molecules that can be transported in the aqueous medium of blood only in combination with more or less hydrophilic protein, phospholipid and unesterified cholesterol. As in cell membranes, lipid-protein binding in plasma lipoproteins appears to depend mainly on hydrophobic bonding between phospholipid fatty acid and hydrophobic areas of the protein (Jackson et al. 1976). Chylomicrons and VLDL are large triglyceride-rich lipoproteins with diameters of 750-6000 Å and 200-750 Å respectively. A 2-3 Å thick monolayer of mainly phospholipid and protein surrounds core of triglyceride

Preface

As will be apparent to the reader Richard J. Havel has been important to me. The basic studies for this doctoral thesis (Fargeman & Havel 1975; Mjss et al 1975; Fargeman et al 1975) were performed in 1972-1974 during a research apprenticeship in his laboratory in the Cardiovascular Research Institute, University of California, San Francisco. He was a master teacher and I am very grateful to him. Tsunako Sata, Agne Frank, Leila Kotite, Ole D. Mjss, Robert L. Hamilton, John P. Kane, Christopher J. Fielding, Phoebe E. Fielding, Philip J. Bart, Teizo Sata, Philip Frost, M. John Chapman, J. H. Fargeman and many others then in San Francisco contributed significantly to these studies with technical assistance, collaboration, advice, discussion and agreeable background noise.

In Copenhagen, Hans Meinertz had introduced me to the lipoprotein field in 1969 and Anders Tybjaerg-Nielsen has maintained me there. I am deeply indebted to them both. Other people of importance have been and are Jørgen J. Jacobsen, Finn Damgaard-Pedersen, Johan Georg, Kurt Iversen, Ulla Vonger, J. H. Fargeman and many others.

The U.S. Public Health Service (grant PO5 TW 1939), the Danish Heart Foundation and the Danish Medical Research Council supported my work in San Francisco. The following review was prepared in part during tenure of a separate grant from the Danish Medical Research Council.

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Table 1 Approximate composition of human plasma lipoproteins (weight percent)

	HDL	LDL	VLDL	chylomicrons
Density	1.043-1.21	1.006-1.043	< 1.006	< 1.006
triglyceride	7	8	50	84
cholesteryl ester	15	40	16	4
unesterified chol	3	10	8	2
phospholipid	25	22	18	8
protein	50	20	10	2

hepatic and intestinal lipoproteins

The apoproteins or the protein components of lipoproteins have been the object of intense study during the past fifteen years (Shore & Shore 1972; Eisenberg & Levy 1973; Jackson et al. 1974). Eight apoproteins occurring in normal lipoproteins have been partially characterized (Table II). They are identified either by their carboxy terminal amino acid or by the system of nomenclature proposed by Alapovici et al. (1964). According to the latter the major apoproteins of HDL are designated apo A I and apo A-II, the major apoprotein of LDL is apo B and the major apoproteins of VLDL and chylomicrons are apo B and the C I, C II and C III apoproteins. This nomenclature is usually used independently of the underlying concept of lipoprotein families (Alapovici 1972). A "lipoprotein family" according to Alapovici and his associates and as opposed to the way in which the same term is employed for example by Eisenberg & Levy (1973) is a system of lipoproteins characterized by the presence of at least one apolipoprotein or its constitutive polypeptide. For example the lipoprotein C family is defined by the presence of apo C or its constitutive polypeptides apo C I, apo C II and apo C III. Although mainly present in the very low density range the lipoprotein C family extends into the high density range also.

This concept is fundamentally at odds with the generally accepted assumption that the density classes of lipoproteins separated by ultracentrifugation are approximately physiologically meaningful entities. According to the latter concept as it has developed in most of the lipoprotein literature the apoproteins as well as the lipid components of lipoproteins may be variable. The C group of apoproteins especially is known to exchange between chylomicrons and VLDL on the one hand and HDL on the other during the process of chylomicron and VLDL catabolism. In contrast apo B seems to be an integral component of chylomicrons, VLDL and LDL. Apo A I and apo A-II are similarly integral components of HDL.

Some apoproteins also serve as cofactors for enzymes active in lipoprotein metabolism. The HDL associated enzyme LCAT catalyzes the transfer of fatty acid from lecithin to cholesterol. Apo A-I may participate in the reaction by binding to the substrate product (Friedberg et al. 1972). Apo D ("thin line peptide

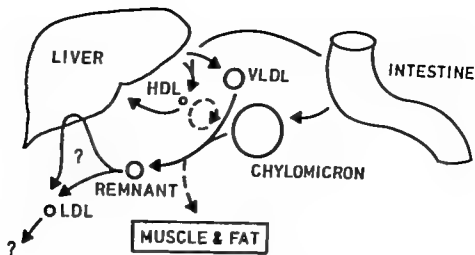


Fig 1 Diagram of the probable major pathways of plasma lipoprotein metabolism

and some cholesteryl ester (Havel 1975). The surface layer also contains most of the unesterified cholesterol (Kilversmit 1968; Sata et al 1972).

Recent studies suggest the same basic model for the small and denser LDL (diameter 170 - 260 Å). The hydrophobic core of these lipoproteins however is mainly cholesteryl esters (Deckelbaum et al 1975). HDL (diameter 60 - 100 Å) also have a core of cholesteryl esters. The various structural models proposed for HDL differ mainly with respect to the interrelationships of the surface constituents (Jackson et al 1976).

Table I presents approximate average compositional data based on figures in a review by Skipski (1972). Chylomicrons and VLDL vary greatly in size and the triacylglyceride content decreases with decreasing particle size. The main phospholipid is lecithin. Sphingomyelin and lysophosphatidylcholine account for significant but smaller proportions. Note that the ratio of esterified to unesterified cholesterol increases with increasing lipoprotein density.

Chylomicrons and VLDL both carry enough triglyceride to have densities less than 1.006 g/ml. An etymologically correct definition of chylomicrons as chylous particles of intestinal origin is sometime used to differentiate them from VLDL with a hepatic origin (Mjøs et al 1975). Usually however these lipoprotein classes are defined operationally according to their flotation rate at $d \leq 1.063$ in the analytical ultracentrifuge. Chylomicrons thus have S_f values greater than 400 and VLDL have S_f values of 20 - 400. In preparative ultracentrifugation 10^6 g x min at $d \leq 1.006$ is usually employed to separate chylomicrons from VLDL. According to the operational definition which will be used in the following the intestine produces both chylomicrons and VLDL whereas the liver normally produces only VLDL. This definition does not preclude important structural and metabolic differences between

Tabl. I Approximate composition of human plasma lipoproteins (weight percent)

	HDL	LDL	VLDL	chylomicrons
Density	1.063-1.21	1.006-1.063	< 1.006	< 1.006
triglyceride	7	8	50	84
cholesteryl ester	15	40	16	4
unesterified chol	3	1	8	2
phospholipid	25	22	10	8
protein	50	20	10	2

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Some apoproteins also serve as cofactors for enzymes active in lipoprotein metabolism. The HDL-associated enzyme LCAT catalyzes the transfer of fatty acid from lecithin to cholesterol. Apo A-I may participate in the reaction by binding to the cholesteryl ester product (Viding et al 1977). Apo B (this line peptide

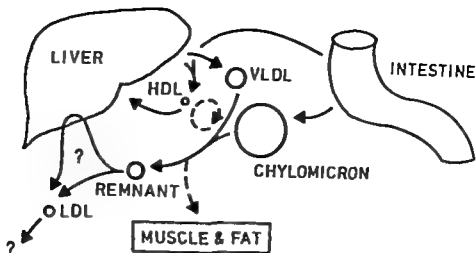


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and VLDL. It has been difficult to study because it tends to aggregate in aqueous media when delipidated. Estimates of molecular weight have ranged from about 255,000 (Smith et al 1972) to about 26,000 (Kane et al 1970) and it may be composed of different subunits. Apo B is an essential structural component of chylomicrons, VLDL and LDL. These lipoproteins are absent from the plasma of patients with abetalipoproteinemia, a rare disease in which apo B synthesis is defective (Gotto et al 1971). Eisenberg & Levy (1973) suggest that apo B bound to phospholipid and cholesterol constitutes structural nuclei common to chylomicrons, VLDL and LDL.

Beside this structural role, apo B may interact with the cell surface receptor mediating LDL catabolism to be discussed later. If so, this interaction is not specific for apo B, since HDL, an abnormal lipoprotein appearing in the plasma of cholesterol-fed dogs and swine binds to the receptor although it contains no apo B (Berse et al 1976).

Synthesis of lipoproteins

Only the liver and small intestine synthesize plasma lipoproteins (Margolis & Capozzi 1972; Hamilton 1972). During absorption of dietary fat, the small intestine secretes large triglyceride-rich lipoproteins (chylomicrons) into the intestinal lymphatic vessels. In the postabsorptive and fasting states, it secretes VLDL whose lipid components come from non-dietary sources, mainly bile and gastrointestinal mucosal sheddings (Gangl & Ockene 1973). The small intestine thus seems capable of secreting endogenous triglyceride-rich lipoproteins, but the liver contribution to the VLDL pool of plasma is generally thought to be larger. The triglyceride incorporated into hepatic VLDL are derived mainly from fatty acids extracted from plasma (Havel 1961; Havel et al 1970 (a); Barter et al 1972) but also depending on diet, drugs and the state of the liver from *de novo* synthesis of fatty acids from hepatic fat stores and from fatty acids originating in the triglyceride of VLDL and chylomicron remnants.

The liver and small intestine also synthesize HDL. Studies in rats indicate that both intestinal HDL and triglyceride-rich lipoproteins are secreted with apo A-I but without C apoproteins. The latter enter the circulation in hepatic HDL and perhaps VLDL (Windemuller et al 1973; Berbert et al 1974; Noel & Rubinstein 1974). Some of the HDL gain access to the intestinal lymph where partial transfer of the C apoproteins to intestinal lipoproteins occurs. The similarity of the metabolism of hepatic and intestinal triglyceride-rich lipoproteins (Brunzell 1973; Faergeman & Havel 1973; Nijss et al 1973) may largely be due to this transfer.

Within the cell, the apoproteins and lipids of VLDL are synthesized and assembled in ribosomes attached to the endoplasmic reticulum. The lipoproteins acquire small amount of carbohydrate during the latter part of their transport through costochondriac system of channels of the rough endoplasmic reticulum, the smooth endo-

and apo C-I (Garner et al 1972) have also been proposed as LCAT cofactors (Shulman 1974; Gjolissen & Gustafson 1974)

Table II Apoproteins of human plasma lipoproteins

Density	HDL 1.063-1.21	LDL 1.006-1.063	VLDL < 1.006	chylomicrons < 1.006
major apoproteins	A-I A-II	B	*A-I B C-I C-II C-III §E	*A-I B C-I C-II C-III E
minor apoproteins	C-I C-II C-III D E			
trace apoproteins	B	C-I C-II C-III	A-I A-II	A-I A-II

*Only in intestinal lipoproteins

§Apo-E arginine-rich apoprotein

The E apoproteins distribute themselves between the triglyceride-rich lipoproteins and HDL according to the concentrations of these lipoproteins in plasma (Eisenberg & Levy 1975). Their structural functions seem to be unimportant since intestinal VLDL and HDL are secreted without them. Apo C-II is a cofactor for lipoprotein lipase (Savel et al 1970 (b); LaRosa et al 1970) but the possible metabolic functions of apo C-I and C-III are not known for certain. Although as mentioned above apo C-I is one of several candidates for an LCAT cofactor role it has also been proposed as cofactor for lipoprotein lipase (Wiand et al 1975). Perhaps by way of compensation for this uncertainty both apo C-I and C-III were honored a few years ago by having their amino acid sequences worked out (Braver et al 1972; Shulman et al 1972).

The arginine-rich apoprotein (Shore et al 1974) or apo E is also a major component of normal VLDL (Kane et al 1975) and can be demonstrated in HDL. It is particularly prominent in remnant lipoproteins of the rat (Mjøs et al 1975) and in the abnormal beta migrating VLDL of patients with dysbetalipoproteinemia (type III^h hyperlipoproteinemia) (Havel & Kane 1973). Since the latter lipoproteins also are thought to be remnants of triglyceride-rich lipoproteins the arginine-rich apoprotein may be intimately involved in remnant metabolism (Havel 1975).

Apo B is the only major apoprotein of LDL and a major apoprotein of chylomicrons

coll agrees (Goodman 1965) Redgrave (1970) injected lymph chylomicrons into un-anesthetized rats and found 10 minutes later that the liver had removed 83% of the injected cholesteryl esters but only 20% of the injected triglyceride. Functional hepatectomy caused accumulation in the plasma of triglyceride-depleted lipoproteins that Redgrave called remnants. When remnants were injected into intact rats they were removed very rapidly from the plasma by the liver. Bergman et al (1971) measured arterio-venous differences across the portal drained viscera, the liver and the lower body of sheep and dogs. They found that the lower body selectively removed triglyceride fatty acid from infused lymph chylomicrons but did not measurably take up cholesteryl esters. Measurements in the sheep showed that the liver and this was drained by the portal vein accounted for 85 and 18% of cholesteryl ester removal respectively.

The experiments cited above were all conducted with intestinal lipoproteins. Nye et al (1975) studied formation of remnants from VLDL of blood plasma mainly of hepatic origin as well as from triglyceride-rich lipoproteins of intestinal lymph. Essentially similar results were obtained when the lipoproteins were injected into supradiaphragmatic rats anesthetized animals whose circulation below the diaphragm had been stopped by ligatures around the aorta and the inferior caval and portal veins.

Data from one of two similar experiments are presented in Table III. Biologically labeled plasma VLDL were obtained from rats injected with (1-²⁻³H)cholesterol and (1-¹⁴C)palmitate 6 and 3 hours respectively before bleeding. Thirty minutes after injection into supradiaphragmatic rats the ratio of recoveries of injected triglyceride ¹⁴C to esterified cholesterol ³H in $d < 1.063$ lipoproteins had fallen to 25-28 indicating that remnants depleted of triglyceride relative to cholesteryl esters had been formed. As more triglyceride was removed more esterified cholesterol ³H appeared in the LDL range ($d \approx 1.063-1.063$). For reasons to be discussed later the esterified cholesterol ³H gradually appearing in $d > 1.063$ lipoproteins probably represented esterification by LCAT of simultaneously injected unesterified cholesterol ³H. Remnants may thus be formed throughout the whole $d < 1.063$ spectrum of lipoproteins under these experimental conditions. The structural details of the $d < 1.063$ part of this spectrum were studied more closely by intraperitoneal injection of 4-amino-2-pyrazolopyrimidine which in situ blocks VLDL secretion from the liver. This permitted study of remnants of chylomicron and VLDL injected via the caval stump and uncontaminated by the supradiaphragmatic animal's own VLDL. The remnants appeared by electron microscopy and chemical composition studies including polyacrylamide gel electrophoretic separation of apoproteins to reveal the fundamental structure of their precursors. A core of cholesteryl esters and triglyceride was surrounded by monolayers of unesterified cholesterol phospholipid and apoproteins. As triglyceride was removed cholesteryl esters assumed more dominant roles as core materials and of the surface components phospholipids. It must be noted that in serum the triglyceride-rich lipo-

plasmaic reticulum and the Golgi apparatus (Havel 1975; Eisenberg & Levy 1975) The newly formed VLDL are released from the Golgi apparatus into the space of Disse in the liver or into the lymphatic vessels of the intestine Hepatic VLDL may acquire C apoproteins either immediately prior to this release from the hepatocyte (Mastcruck & Rubinstein 1975) or when they encounter hepatogenous HDL (Havel 1975)

The intracellular events involved in HDL formation are not as well known HDL may be secreted with an incomplete complement of lipid as discs composed of two layers of apoprotein-phospholipid between which a lipid core of cholesteryl esters is formed in the plasma by LCAT (Porte et al 1974; Hamilton et al 1976)

As will be discussed below LDL are probably catabolic products of the triglyceride-rich lipoproteins and thus may not be independently secreted from the liver or small intestine

Catabolism of triglyceride-rich lipoproteins

The basis for our current understanding of the catabolism of the triglyceride-rich lipoproteins was established in the 1950s (Fredrickson & Gordon 1958) Chylomicrons and VLDL could be shown to be degraded to progressively denser lipoproteins both in vitro when they were incubated with postheparin plasma and in vivo after heparin injection Pierce (1954) injected lipoproteins of different densities into rabbits and found by analytical ultracentrifugation that lipoproteins of high S_f values (chylomicrons and VLDL) were degraded to lipoproteins of low S_f values including the S_f 5-15 class (LDL) and that the reverse process did not take place Since then a large number of radioisotope studies of the apoprotein (Gitlin et al 1958; Fidge & Foxman 1971; Bilheimer et al 1972; Eisenberg et al 1973; Eisenberg & Rachmilewitz 1973; Paerzeman et al 1975; Sigurdsson et al 1975) the triglyceride (Havel et al 1962; Quarfordt et al 1970; Barts & Metel 1972) and the cholesteryl ester components (Loasow et al 1962; Barter 1974; Paerzeman & Havel 1975) of chylomicrons or VLDL have confirmed the direction and sequential nature of the process

The Remnant Hypothesis

Biggs (1957) demonstrated in the rat that triglyceride and cholesteryl esters of chyle lipoproteins were metabolized differently Whereas no hepatic tissue removed most of the triglyceride component the liver removed and apparently hydrolyzed the cholesteryl esters Significantly Biggs suggested that the latter process occurred last This concept was extended by Mellin et al (1963) who found that complete or partial exclusion of the liver from the circulation of the dog substantially delayed removal of unesterified and esterified cholesterol of lymph chylomicrons relative to the triglyceride component The metabolism of the cholesteryl ester component in the rat liver was extensively studied by Goodman and his

colleagues (Goodman 1965) Badgrave (1970) injected lymph chylomicrons into un-anesthetized rats and found 10 minute later that the liver had removed 83% of the injected cholesteryl esters but only 20% of the injected triglyceride. Functional hepatectomy caused accumulation in the plasma of triglyceride-depleted lipoproteins that Badgrave called remnants. When remnants were injected into intact rats they were removed very rapidly from the plasma by the liver. Bergman et al (1971) measured arterio-venous differences across the portal drained viscera, the liver and the lower body of sheep and dogs. They found that the lower body selectively removed triglyceride of fatty acid infused lymph chylomicrons but did not measurably take up cholesteryl esters. Measurements in the sheep showed that the liver and tissues drained by the portal vein accounted for 85 and 18% of cholesteryl ester removal respectively.

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Data from one of two similar experiments are presented in Table III. Biologically labeled plasma VLDL were obtained from rats injected with (1,2-³H)cholesterol and (1-¹⁴C)palmitate 6 and .5 hours respectively before bleeding. Thirty minutes after injection into supradiaphragmatic rats the ratio of recoveries of injected triglyceride ¹⁴C to esterified cholesterol ³H in $d < 1.063$ lipoproteins had fallen to 25-28% indicating that remnants depleted of triglyceride relative to cholesteryl esters had been formed. As more triglyceride was removed more esterified cholesterol ³ appeared in the LDL range of $d 1.063-1.063$. For reasons to be discussed later the esterified cholesterol ³ gradually appearing in $d > 1.063$ lipoproteins probably represented esterification by LCAT of simultaneously injected unesterified cholesterol ²H. Remnants may thus be formed throughout the whole $d < 1.063$ spectrum of lipoproteins under these experimental conditions. The structural details of the $d < 1.063$ part of this spectrum were studied more closely. Prior intraperitoneal injection of 4-aminopyrrolisopropylamine which intra alia blocks VLDL secretion from the liver permitted study of remnants of chylomicrons and VLDL injected via the caval stump and uncontaminated by the supradiaphragmatic animal's own VLDL. The remnants appeared by electron microscopy and chemical composition studies including polyacrylamide gel electrophoretic separation of apoprotein to retain the fundamental structure of their precursors. A core of cholesteryl esters and triglycerides was surrounded by a monolayer of unesterified cholesterol phospholipid and apoproteins. As triglyceride was removed cholesteryl esters assumed more dominant roles as core material and, of the surface components phospholipids and C apoproteins seemed to leave the triglyceride-rich lipo-

Tabl. III Distribution of Radioactivity in Lipids of Lipoprotein Fractions after Injection of Doubly Labeled VLDL into Supradiephragmatic Rats

Time after injection	Esterified ^3H chol sterol as percent of that injected					Injected ^{14}C recovered in			Recovery ratio $\frac{\$}{\text{}}$
	$\bar{d} < 1.006$	\bar{d}	\bar{d}	\bar{d}	Total in	$\bar{d} < 1.006$	$\bar{d} > 1.063$		
	$\bar{d} < 1.006$	$1.006-1.019$	$1.019-1.063$	$1.063-1.083$	$\bar{d} < 1.063$	$\bar{d} < 1.006$	$\bar{d} > 1.063$		
min						TCFA	FFA		
2.5	66.9	4.2	1.2		72.3	62.8	3.0		0.94
2.5	64.3	4.9	3.7		72.9	37.6	18.6		0.58
5	78.5	2.0	1.4		81.9	60.7	8.8		0.77
5	61.4	5.1	5.1		71.6	29.2	10.3		0.48
15	63.0	7.4	10.2		80.6	26.6	6.2		0.42
15	33.6	14.0	27.7		75.3	4.4	1.4		0.13
30	53.7	8.6	11.2		73.5	13.2	0.8		0.25
30	64.3	5.8	5.4		75.5	17.7	1.1		0.28

Calculated from radioactivity contained in blood plasma. Plasma volume was determined for each rat from dilution of ^{125}I labeled human serum albumin.

$\frac{\$}{\text{}}$ Ratio of ^{14}C triglyceride fatty acid to esterified ^3H chol sterol in $\bar{d} < 1.006$ lipoproteins (ratio in injected material normalized to unity)

TCFA triglyceride fatty acid FFA free fatty acids
(Reproduced with permission of J. Clin. Invest.)

proteins Whereas apoproteins identified as the arginine-rich peptide and in the case of intestinal lipoproteins apo A-I seemed to remain apo B could in the case of VLDL remnants be shown to be the most constant component

These rat studies suggested that hepatic and intestinal triglyceride-rich lipoproteins are catabolized by similar if not identical mechanisms as far as the lipoprotein lipase step Similar conclusions were drawn by Brunzell et al (1973) on the basis of human studies On the other hand Wieland et al (1973) have presented preliminary evidence that human intestinal and hepatic triglyceride-rich lipoproteins are catabolized by different apo C I and apo C II activated lipoprotein lipases respectively

In the supradiaphragmatic rat the lipoprotein lipase of adipose tissue skeletal muscle and heart may complete the conversion of triglyceride-rich lipoproteins to LDL (Table III) Isolated rat heart studies have shown that as triglyceride is removed, the remnant lipoproteins become progressively poorer substrate for lipoprotein lipase (Fielding & Higgins 1974) possibly due to the concomitant loss of the lipoprotein lipase cofactor apo C II demonstrated in studies of VLDL incubated with post-heparin plasma (Kisenberg & Reichlewitz 1973) In the intact animal remnant lipoproteins are vulnerable to removal by the liver before serial passages through extra-hepatic tissues allow lipoprotein lipase to degrade them completely to LDL The extent of this degradation may be a complex function of lipoprotein structure lipoprotein interactions lipoprotein lipase efficiency remnant affinity for liver cells and possibly other metabolic parameters Not surprisingly therefore it varies importantly between animal species as will appear later In the following the fate of the various components of chylomicrons and VLDL will be considered, with emphasis on cholesteryl esters and apo B

Phospholipids and unesterified cholesterol Rubenstein & Rubenstein (1972) found that lecithin and sphingomyelin of rat VLDL transferred mainly to HDL both in vivo and in vitro Small amounts of both phospholipids but relatively more sphingomyelin were also recovered in LDL Whereas C apoproteins in the same experiments apparently exchanged between VLDL and HDL the phospholipids seemed to transfer unidirectionally This may be due at least in part to the action of LCAT (Worms 1971 Glomset 1972) Located on HDL this enzyme catalyzes the transfer of an acyl group from the 3 position of lecithin to the 3 position of cholesterol Whereas the resulting lysolecithin becomes associated with $d > 1.21$ proteins of plasma the esterified cholesterol enters the putative core of the HDL particle (Faergeman & Kavel 1973) By favouring net transfer of both surface constituents lecithin and cholesterol from the triglyceride-rich lipoproteins to HDL LCAT acts to reduce surface material as core triglyceride is removed by lipoprotein lipase (Schwenker Adams 1970) The basic structure of the triglyceride-rich lipoproteins is therefore retained in their remnants (Moss et al 1973) Recent in vitro studies suggest that the phospholipase activity associated with lipoprotein lipase and hepatic triglyceride

ride hydrolase may be as important as LCAT in removing phospholipids from triglyceride-rich lipoproteins (Eisenberg & Schurr 1976). In the intact animal molecular exchange of phospholipids and unesterified cholesterol between triglyceride-rich lipoproteins and various cell membranes may interact significantly with these enzymatic processes.

C apoproteins Several groups of investigators have demonstrated the ready exchange of C apoproteins between triglyceride-rich lipoproteins and HDL (Eisenberg & Levy 1975; Rubenstein & Rubinstein 1972; Havel et al 1973; Paargaman et al 1975). In association with the relatively slowly turning over pool of HDL, the C apoproteins constitute a circulating supply of cofactors readily available to the fluctuating requirements of triglyceride-rich lipoprotein catabolism.

Triglyceride After hydrolysis of the ester bond by lipoprotein lipase in adipose tissue, skeletal muscle or heart (Oliverson & Belfrage 1965; Havel 1965; Nikkila 1969), the fatty acids are either taken up by the parenchymal cells or, as illustrated in Table III, released into the blood stream where they attach to albumin for rapid transit to other tissues, including the liver. Whereas extra-hepatic tissues remove only small amounts of the other lipid constituents of chylomicrons and VLDL, hepatic uptake of triglyceride fatty acids probably occurs as part of hepatic remnant removal (Havel 1965; Elvesson et al 1965; Belfrage 1968). Between 10 and 30% of chylomicron and VLDL triglyceride may reach the liver, but this varies between animal species and depends on the nutritional state of the animal (Havel et al 1962; Havel 1965; Bergman et al 1971; Paargaman & Havel 1975; Milson-Ehle 1975). An undetermined amount of chylomicron or VLDL triglyceride may transfer to a rapidly turning over HDL subfraction (Barter & Connor 1975) whose ultimate fate is uncertain.

Injection of heparin causes the release into plasma of both lipoprotein lipase and a hepatic triglyceride hydrolase. Despite speculation about possible role in hydrolyzing residual triglyceride and phospholipid in remnant lipoproteins (Eisenberg & Levy 1975), the function of the hepatic enzyme is unknown. Its contribution to post-heparin lipase activity of human plasma, increased by treatment with oxandrolone, a synthetic steroid (Kohnholtz et al 1975). This may explain the poor correlation between triglyceride removal efficiency and total post-heparin lipase activity observed in oxandrolone-treated patients with exogenous (Paargaman & Damgaard-Pedersen 1973) and endogenous hypertriglyceridemia (Glueck et al 1973).

Cholesterol Discussion of the metabolism of unesterified and especially the esterified cholesterol of chylomicrons and VLDL requires consideration of the sources of plasma cholesteryl esters and their transfer between lipoproteins. The mucosal cells of the small intestine contain enzymes capable of synthesizing the

major part of absorbed dietary cholesterol before its incorporation into VLDL and chylomicrons (Goodman 1965; Endel et al 1973). The source of cholesteryl esters in VLDL of hepatic origin however may vary between species. Rat VLDL derive their cholesteryl esters mainly from hepatic cholesterol esterifying enzyme (i.e. acyl coA:cholesterol acyltransferase) (Goodman 1965) located in the Golgi apparatus where VLDL formation is completed (Assmann et al 1974). Rabbit VLDL may also especially during cholesterol feeding derive most of their cholesteryl esters from the liver (Rose 1972). Cholesterol esterifying enzymes are apparently not present in human liver however (Stokke 1972) and cholesteryl esters of human plasma not derived from the intestine have all been thought to be formed in plasma by LCAT (Glosset 1972). The question of possible hepatic contribution to cholesteryl esters of human plasma is nevertheless still open (Barts 1974).

In contrast to the case in the human the composition of rat plasma cholesteryl esters varies between lipoprotein classes (Gidez et al 1965; Faergeman & Havel 1975). Whereas HDL cholesterol is esterified mainly with 2 to 4 and 16 to 18 fatty acids reflecting LCAT mediated transfer from lecithin VLDL cholesteryl esters contain mostly 18:1, 18:2 and 16 acids reflecting hepatic origin (Gidez et al 1967). LDL cholesteryl ester composition is intermediate. This heterogeneity facilitated studies of transport in plasma of rat cholesteryl esters (Faergeman & Havel 1975).

As in recent studies previous studies of cholesteryl ester metabolism in triglyceride-rich lipoproteins had been performed with intestinal lipoproteins (Goodman 1962; Quarfordt & Goodman 1967; Quarfordt & Goodman 1969; Stein et al 1969). Faergeman & Havel (1975) obtained biologically labeled plasma VLDL from rats injected 6 hours previously with ^3H -cholesterol. About 30% of the labeled cholesterol was in esterified form. When injected into recipient rats most of the esterified and unesterified cholesterol ^3H was rapidly taken up in the liver where the cholesteryl esters subsequently were hydrolyzed (Tables IV and V). A small fraction of the injected esterified cholesterol ^3H about 3% appeared via lipoproteins of intermediate density ($d = 1.006-1.10$) in LDL ($d = 1.019-1.063$) of plasma. Argmentation thin layer chromatography studies showed that the distribution of cholesterol ^3H in the various parts of LDL and the liver was the same as that of the injected VLDL. In contrast distribution of cholesterol ^3H gradually appearing in HDL reflected esterification by LCAT of the simultaneously injected unesterified cholesterol ^3H . Since the mass distribution of cholesteryl esters in LDL as determined by gas liquid chromatography was intermediate between that of VLDL and HDL it was apparent that LDL esters had dual origin transfer from VLDL and LCAT esterification presumably on HDL with subsequent transfer to LDL.

The extensive hepatic removal from plasma of both cholesteryl esters component and unesterified cholesterol surface component suggested endocytosis of the whole VLDL remnant. Early experiments in these studies showed that small structural change in the injected VLDL could markedly affect this hepatic uptake mechanism. As in Goodman (1962) work with chylomicrons the ratio of esterified

Table IV Esterifi d ³H-Cholesterol as Percentage of Injected VLDL Esterifi d ³H-Cholesterol

Time after inj ction	Plasma lipoproteins				
	$\bar{d} < 1.006$ (n = 8)	$\bar{d} 1.006-1.019$ (n = 8)	$\bar{d} 1.019-1.063$ (n = 8)	$\bar{d} > 1.063$ (n = 6)	Liver (n = 8)
min					
5	37.9 (12.3)*	2.2 (1.1)	1.2 (0.7)	1.3 (0.3)	50.8 (12.9)
10	20.2 ^S	1.9	1.5	1.5	64.5
15	8.8 (6.8)	2.5 (1.3)	1.6 (1.2)	1.4 (0.3)	70.9 (10.2)
30	1.5 (0.9)	2.1 (1.6)	2.3 (1.7)	1.7 (0.5)	31.2 (8.8)
60	1.6 (0.5)	1.4 (0.9)	3.3 (1.8)	3.2 (1.2)	12.4 (2.4)

Mean and SD values calculated for plasma volume = 4% of body wt

S_n 2

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Tabl. V Free ^3H -Cholesterol as Percent of Injected VLDL Free ^3H -Cholesterol

Time after injection	Plasma lipoproteins						Live (%)
	$\bar{d} < 1.006$ (%)		$\bar{d} 1.006-1.019$ (%)	$\bar{d} 1.19-1.063$ (%)	$\bar{d} > 1.063$ (%)		
min							
5	26.7 (1.7)	1.4 (.8)		4.1 (1.1)	6.9 (.8)	53.5 (7.5)	
10	13.7 ⁵	1.1		3.4	4.9	59.9	
15	4.4 (3.8)	9 (.5)		1.9 (.5)	2.8 (.8)	74.2 (9.9)	
20	0.9 (.4)	6 (.4)		1.6 (.4)	2.3 (.3)	91.9 (14.)	
60	1.5 (.6)	6 (.4)		1.8 (.7)	3.3 (.4)	82 (8.3)	

Mean and SD values calculated for plasma volume 4% of body wt

5 2

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Table IV Esterified ³H-Cholesterol as Percentage of Injected VLDL Esterified ³H-Cholesterol

Time after injection	Plasma lipoproteins				
	$\bar{d} < 1.006$ (n = 8)	$\bar{d} 1.006-1.019$ (n = 8)	$\bar{d} 1.019-1.063$ (n = 8)	$\bar{d} > 1.063$ (n = 6)	Liver (n = 8)
<u>min</u>					
5	37.9 (12.3)*	2.2 (1.1)	1.2 (0.7)	1.3 (0.3)	50.8 (12.9)
10	20.2 [§]	1.9	1.5	1.5	64.5
15	8.8 (6.8)	2.5 (1.3)	1.6 (1.2)	1.4 (0.3)	70.9 (10.2)
30	1.5 (0.9)	2.1 (1.6)	2.3 (1.7)	1.7 (0.5)	31.2 (8.8)
60	1.6 (0.5)	1.4 (0.9)	3.3 (1.8)	3.2 (1.2)	12.4 (2.4)

*Mean and SD values calculated for plasma volume = 4% of body wt
[§] n = 2

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The possible importance of cholesteryl ester transfer reactions for transport of cholesterol from non-hepatic tissues to the liver will be considered at the end of this review.

3 apoprotein The heterogeneous origin of the cholesteryl esters of rat LDL contrasts with the finding that the other major LDL component apo B is probably exclusively derived from VLDL (Faergeman et al. 1975). These studies were also based on work with biologically labeled VLDL. The incorporation of intravenously injected ^3H lysine into tetraethylurea insoluble (apo B) and soluble (mainly apo C) apoproteins of VLDL and LDL produced specific activity curves compatible with precursor-product relationship between apo B of VLDL and LDL respectively (Fig. 2). When VLDL labeled in this manner were injected into other rats the fate of apo B closely approximated that of cholesteryl esters: a small amount (about 3%) was recovered in plasma LDL whereas the major part rapidly appeared in the liver where it was slowly degraded (Table V). In these recipient rats the specific activity relationships again indicated that apo B of VLDL could be the sole precursor of LDL apo B. Incubation of labeled VLDL with unlabeled HDL decreased the amount of ^3H due to non-B apoproteins (C apoproteins) from about 35 to 14% by molecular exchange. This incubation procedure did not, as had been the case with erythrocyte incubation, affect the metabolic behaviour of the VLDL in recipient rats.

These studies were in essential agreement with studies of ^{125}I labeled VLDL also conducted in the rat (Eisenberg & Reichle 1973; Reibstein et al. 1973) and supported the usefulness of the latter more extensively employed techniques in metabolic experiments of this kind (Fidge & Poulis 1974). Moreover they were the first along with simultaneously published studies of human lipoproteins (Sigurdsson et al. 1975; Phair et al. 1975) to document with specific activity data the concept of precursor-product relationship between apo B of VLDL and LDL. Both human and rat LDL thus seem to originate in plasma VLDL. However, the extent to which VLDL apo B is converted to LDL apo B or rapidly removed in the liver differs markedly in these two species as will be discussed below.

Bypass uptake in the liver

The perfused rat liver can remove from the perfusate in vitro labeled cholesteryl esters of intact intralipid chylomicrons (Quarfordt & D.S. Goodman 1969) or intact serum lipoproteins (E.D. Goodman & Lequire 1975). And the latter authors suggested the exchange of cholesteryl esters between lipoproteins and hepatocytes is physiologically important. However, the rate of uptake and subsequent hydrolysis is faster when the perfused liver is presented with lipoproteins that have passed through non-hepatic tissues (Quarfordt & D.S. Goodman 1969; Sherrill & Dietschy 1976). This strengthens the impression gained from studies with intact animals that hepatic removal of VLDL or chylomicron components occurs during uptake of intact remnant

to unesterified chol terol ^3H in donor VLDL could be increased substantially by incubation of the lipoproteins with erythrocytes containing exchangeable unlabeled unesterified cholesterol. The chol sterol ^3H of the incubated VLDL however appeared in the liver of recipient animals much more rapidly than that of unincubated VLDL and more extensive hepatic uptake of ^{14}C triglyceride fatty acid occurred. Incubated VLDL contained more unesterified cholesterol and less phospholipid than the unincubated lipoproteins. Small alterations of these surface components may thus dramatically affect the manner in which the lipoprotein is metabolized.

These cholesteryl ester studies indicated that in the case of chylomicrons the remnants of rat VLDL are rapidly taken up in the liver and that only a minor part completes the conversion to LDL. Moreover they showed that rat LDL cholesteryl esters are apparently derived from both VLDL and HDL associated LCAT. Glomset (1972) has suggested that cholesteryl esters of HDL may be transferred to the triglyceride-rich lipoproteins in exchange for lecithin unesterified cholesterol and a small amount of triglyceride. The mechanism of this transfer has been the subject of different incubation studies. In a preliminary report Glomset et al (1974) proposed that LCAT mediates transfer of the arginine-rich apoprotein from HDL to VLDL in conjunction with cholesteryl esters. This agrees well with findings that the arginine-rich apoprotein is prominent in rat VLDL and chylomicron remnants (Mjøs et al 1975) and in human dysbetalipoproteinemia (Havel & Kane 1973).

Recently Olofsson (1975) found that incubation of a HDL subfraction obtained by hydroxyl apatite column chromatography broke up the lipoprotein in a lipid poor fraction ("HDL^{inf}") and a fraction ("HDL^{sup}") composed mainly of cholesteryl esters coupled to a polypeptide different from the recognized apoproteins of HDL. The author suggested that this polypeptide transfers cholesteryl esters from HDL to VLDL in vivo. Whether HDL subfractions isolated by in vitro techniques of this kind may correspond to the rapidly turning over HDL subpool suggested by in vivo studies (Barter 1974; Barter & Connor 1975; Barter et al 1977) is unknown.

The concept of cholesteryl ester acquisition by triglyceride-rich lipoproteins and their remnants is not readily consistent with all available data. However, using calculations based on median molecular weights of subfractions of the S_f 20-400 range Eisenberg et al (1973) found in a study of hyperlipidemic humans that in one subject the esterified cholesterol content of each particle decreased with increasing particle density. In another subject it remained constant. Similarly Eisenberg & Rachellewits (1975) calculated that in vitro conversion of rat VLDL to remnants by postheparin plasma involved loss of all components except apo B. In contrast Mjøs et al (1975) employing calculations based on molecular weights derived from chemical composition studies and electron microscopy determination of particle sizes found that the cholesteryl ester content per particle increased as VLDL were degraded to remnants. This increase seems to depend on the availability of HDL, since it did not occur in 4 aminopyrazolepyrimidin treated rats in which HDL levels were low.

The possible importance of cholesteryl ester transfer reactions for transport of cholesterol from non-hepatic tissues to the liver will be considered at the end of this review.

apoproteins The heterogeneous origin of the cholesteryl esters of rat LDL contrasts with the finding that the other major LDL component apo B is probably exclusively derived from VLDL (Faergeman et al 1973). These studies were also based on work with biologically labeled VLDL. The incorporation of intravenously injected ^3H -lysine into tetramethylurea insoluble (apo B) and soluble (mainly apo C) apoproteins of VLDL and LDL produced specific activity curves compatible with precursor-product relationship between apo B of VLDL and LDL respectively (Fig. 2). When VLDL labeled in this manner were injected into other rats the fate of apo B closely approximated that of cholesteryl esters: small amount about 3% was recovered in plasma LDL whereas the major part rapidly appeared in the liver where it was slowly degraded (Table VI). In these recipient rats the specific activity relationships again indicated that apo B of VLDL could be the sole precursor of LDL apo B. Incubation of labeled VLDL with unlabeled HDL decreased the amount of ^3H due to non-B apoproteins (C apoproteins) from about 35 to 14% by molecular exchange. This incubation procedure did not as had been the case with erythrocyte incubation affect the metabolic behaviour of the VLDL in recipient rats.

These studies were in essential agreement with studies of ^{125}I labeled VLDL also conducted in the rat (Eisenberg & Reichle 1973; Roberts et al 1973) and supported the usefulness of the latter more extensively employed techniques in metabolic experiments of this kind (Midge & Poulis 1974). Moreover they were the first along with simultaneously published studies of human lipoproteins (Sigurdsson et al 1973; Fair et al 1975) to document with specific activity data the concept of precursor-product relationship between apo B of VLDL and LDL. Both human and rat LDL thus seem to originate in plasma VLDL. However the extent to which VLDL apo B is converted to LDL apo B or rapidly removed in the liver differs markedly in these two species as will be discussed below.

Relevant uptake in the liver

The perfused rat liver can remove from the perfusate in vitro labeled cholesteryl esters of intact intestinal chylomicrons (Quarfordt & D. S. Goodman 1969), intact serum lipoproteins (S. D. Goodman & LeQuire 1975) and the latter authors suggested the exchange of cholesteryl esters between lipoproteins and hepatocytes is physiologically important. However the rate of uptake and subsequent hydrolysis is fast when the perfused liver is presented with lipoproteins that have passed through non-hepatic tissues (Quarfordt & D. S. Goodman 1969; Sherrill & Dietschy 1976). This strengthens the impression gained from studies with intact animal that hepatic removal of VLDL or chylomicron components occurs during uptake of intact reactant

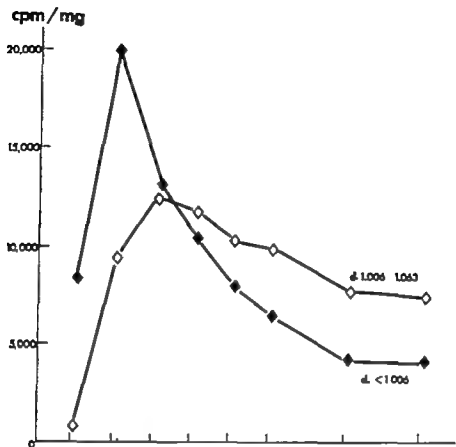


Fig 2 Specific activity of tetramethylurea insoluble apoprotein (po B) of VLDL and LDL of ^3H lysine injected rats. Each point represents pooled lipoprotein from 3 animals. (Reproduced with permission of J clin Invest.)

lipoproteins (Belfrage 1968; Havel 1965; Bergman et al 1971; Stein et al 1969; Faergeman & Havel 1975; Stein et al 1974; Faergeman et al 1975). For example cholesteryl esters and apo B components of VLDL are taken up in the liver to similar extent and at similar rate consistent with uptake of both components in one unit (Faergeman & Havel 1975; Faergeman et al 1975).

Whereas the triglycerides may be hydrolyzed while the remnant is attached to the hepatocyte plasma membrane (Belfrage 1968) the apoprotein may be degraded in secondary lysosomes of the cell interior (Stein et al 1974). Hydrolysis of cholesteryl esters seems to occur at or immediately inside the plasma membrane (Stein et al 1969) possibly in secondary lysosomes (Stein et al 1977).

Control of remnant uptake in the liver is the subject of considerable interest. Although subtle alterations in the unesterified cholesterol and phospholipid surface components of the VLDL precursor may affect the rate of uptake (Faergeman & Havel 1975) a characteristic component of remnants would seem more likely to trigger recognition by the liver. Havel (1975) has suggested that the arginine-rich apoprotein may serve this purpose. After remnant uptake in the liver the arginine-rich apoprotein may recycle into the plasma to become associated with HDL.

Table VI Distribution of ^3H after Intravenous Injection of ^3H -Lysine-labeled VLM. Incubated with HNE to Reduce ^3H in TMO-Soluble Proteins

	Number	Time after injection				
		5 min	15 min	30 min	60 min	
\bar{v} of injected ^3H						
Lipoproteins						
$d < 1.006$	8 (4) 5	33.7 13.4 [†] (34.3 14.4)	12.8 [†] 6.8 (11.9 7.7)	7.1 [†] 3.5 (4.9 2.3)	2.4 [†] 1.2 (1 [†] 1)	
$d 1.006-1.018$	8	2.1 1.1	2.4 1.3	2.0 [†] .8	1 [†] .5	
$d 1.019-1.043$	8 (4)	8 3 (6 3)	2.0 [†] .7 (1.7 .8)	2.6 [†] .7 (2.4 [†] .8)	2.9 [†] .9 (2.7 [†] 1)	
$d 1.043-1.21$	8	3.8 1.8	4.1 2	2.9 1.8	4.1 2.4	
$d > 1.21$	6	1.9 1.4	2.3 [†] 1.2	1.9 [†] 1.5	4 [†] 2.4	
Organs						
Liver	8	36.3 11.8	50.5 5.3	48.5 8.3	40.9 [†] 10.4	
Intestine	4	4 1	8 [†] .6	1.8 1.7	1.0 1.0	
Spleen	2		4		.3	
Kidneys	2		1		1	
Heart	2		3		.2	
Lungs	2		8		1.6	
Total		78	77.5	66.8	59.4	

[†]Based upon plasma volume of 4.5% of body weight

[‡]Mean values ± SD

[§]Numbers in parentheses refer to TMO-insoluble protein as percent of injected TMO-insoluble ^3H 86% of the labeled protein injected was insoluble in TMO

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and $d > 121$ proteins. The hepatocyte surface may possess a remnant specific receptor since evidence presented in abstract form by Sherrill & Dietschy (1976) suggests that remnant uptake in the liver is a specific saturable process.

Transfer of apo B from VLDL to LDL

The appearance of a small amount of radioactivity in LDL protein after ^{14}C leucine injection into squirrel monkeys whose VLDL catabolism had been blocked by Triton treatment suggested (Illingworth 1975) that a small amount of LDL apo B in this species may not be derived from VLDL. However the previously mentioned specific activity data obtained in the rat (Faergeman et al 1975) and human (Sigurdsson et al 1975) are consistent with the general impression that all LDL apo B is derived from VLDL apo B.

On the other hand the extent to which VLDL are converted to LDL varies importantly between species. In the rat the amount of injected VLDL apo B that can be recovered in LDL is substantially less than 10% (Eisenberg & Rachmilewitz 1973; Faergeman et al 1975). This is consistent with a calculation showing the LDL apo B turnover rate (mass/time) to be about 4% of that of VLDL apo B (Faergeman et al 1975). Essentially the same conclusion could be drawn from studies of the metabolism of the cholesteryl ester component of rat VLDL (Faergeman & Havli 1975). VLDL metabolism in the human is very different. Recovery in plasma LDL of simultaneously injected ^{131}I VLDL apo B and ^{125}I LDL apo B into normal human subjects allowed Sigurdsson et al (1975) to calculate by two different techniques that a mean of about 90% of VLDL apo B was converted to LDL apo B. In separate experiments these authors found that the mean turnover rate of apo B in LDL was about 82% of that of VLDL apo B with substantial variation. They concluded that most if not all human VLDL apo B is converted to LDL apo B.

Exactly how uptake of remnants in the liver is related to LDL formation is unknown (Fig 1). One possibility is uptake of all remnant in the liver removal of most of the residual triglyceride and non B apoproteins and release into plasma of the resulting LDL in an amount variable between animals (Havli 1975). Another possibility mentioned previously is uptake in the liver of a fixed proportion of the remnants produced by lipoprotein lipase which completes the conversion to LDL of remnants escaping the liver. In the latter case the liver is not directly involved in LDL formation. Whatever the mechanism the extent of LDL formation from VLDL may determine species characteristic plasma LDL concentrations. In the human most of VLDL is converted to LDL and LDL is very high. The opposite is true in the rat and the guinea pig is intermediate in this respect (Barter et al 1977).

Catabolism of LDL

Recent studies of cell cultures and in intact animals suggest that LDL are catabolized in non-hepatic tissues. The molecular basis for this process has been studied in several laboratories notably that of Brown Goldstein and their co-workers (Brown & Goldstein 1976). Results of experiments with cultures of fibroblasts from normal humans and patients with familial hypercholesterolemia or cholesteryl ester storage diseases have led these authors to propose that binding of LDL to high affinity cell surface receptor initiate the following events. After endocytosis of the lipoprotein and fusion of the endocytotic vesicle with lysosomes the apo B and cholesteryl esters are hydrolyzed by lysosomal enzymes. The liberated cholesterol is reesterified by membrane bound fatty acyl coA cholesterol acyltransferase. Rates of LDL uptake in the fibroblast may control the synthesis of both the LDL receptor and hydroxy methyl glutaryl coA reductase. The latter is the rate limiting enzyme in cholesterol synthesis. Regulation of this enzyme is complex however since it may also depend on rates of cholesterol efflux from the cell (Fogelman et al. 1975; Edwards 1975).

These tissue culture studies are consistent with the finding by Sniderman et al (1974) that functional hepatectomy in pigs increases the fractional catabolic rate of LDL. These authors proposed that the liver somehow serves to prolong the life of LDL. Plasma LDL are in rapid equilibrium with an extravascular mainly hepatic pool of LDL perhaps 20-40% of the size of the intravascular pool. Whether this hepatic pool of LDL is extracellular membrane bound or intracellular is unknown are its possible functions in LDL catabolism (Sniderman et al 1975).

Brown & Goldstein (1976) have proposed that receptor-mediated non-hepatic LDL degradation demonstrated in tissue culture studies is part of an in vivo net transport of cholesterol from the liver and small intestine to other tissues to cover requirements of membrane cholesterol. At present this concept remains speculative and the major site of LDL degradation in vivo is uncertain.

Cholesterol transport to the liver

Unesterified cholesterol exchanges between lipoproteins and cell membranes and Glomset (1972) has suggested that cholesterol esterification by LCAT promotes the transfer of cholesterol from non-hepatic tissue cell to HDL circulating in interstitial fluids. Since the liver may be the major site of HDL catabolism (Kisselberg Levy 1977) an important function of these lipoproteins could be transport of cholesterol to the liver for excretion in bile or conversion to bile acids (Mill Miller 1975). Cultured hepatocytes readily bind HDL (Breslow 1973). The previously discussed transfer of cholesteryl esters from HDL to VLDL chylomicrons or their remnants may influence this transport however.

HDL turnover time in the rat is long about 16 hours measured by following the

apoprotein moiety (Eisenberg & Levy 1975) compared to that of remnants about 7 minutes measured as VLDL components removed by the liver (Faergeman & Havel 1975). Since the content of LCAT derived cholesteryl esters in remnants destined for the liver is unknown transport rates cannot be calculated. However the turnover times do suggest that in the rat substantial amounts of cholesterol from non-hepatic tissues could be transported to the liver by remnants. Whether this also could be the case in the human is uncertain because a remnant pathway to the human liver has not been demonstrated experimentally. The likelihood that human remnant metabolism nevertheless is qualitatively similar to that of experimental mammals (dog, sheep, rat) must be considered with the caution that always applies when extrapolating from one animal species to another.

Concluding remarks

The role of the liver in controlling remnant catabolism and LDL formation, the major *in vivo* sites and control of LDL catabolism, and the control and transport functions of HDL are all important objects for further study. As such they may form the basis for a better understanding particularly of the pathways of plasma cholesterol and phospholipid transport.

A detailed understanding of the overall control of the plasma lipoproteins as a complex system for transporting water insoluble lipids will hopefully explain the various derangements of this system that cause human disease.

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Supplementum 616

Non-invasive detection of critical coronary lesions potentially associated with sudden death

Annex

Mexiletine effect on the monophasic action potential (MAP) of right ventricle in man

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1.

INTRODUCTION

Ed Varonovkas

1.

INTRODUCTION

Ed Varatuckas

Improved survival following coronary artery bypass surgery in patients with main stem disease re-emphasizes the well established relationship between prognosis and the extent and severity of coronary atherosclerotic lesions. Critical degree and localization of the stenosis may highly influence the longevity. This stimulates the search for a possible anatomical correlation with sudden death. Establishment of such a correlation might considerably enhance the possibilities to prevent sudden death by adding coronary bypass surgery to other preventive measures.

There are no absolute methods short of arteriography to visualize obstructive lesions in coronary arteries. The shortcomings of coronary arteriography such as cost, risk of serious complications etc. do not permit, however the use of this invasive procedure as a screening method. The non-invasive methods are therefore highly desirable to define the candidate for coronary arteriography.

The present symposium is devoted to discussions of the evidence suggesting a close association between specified critical coronary lesions and sudden death and also to assessment of the possibilities of predicting such lesions from non invasive investigations.

It is a great pleasure for me to thank the renowned investigators for cooperation in presenting their work at the symposium and their manuscripts for this publication.

One manuscript was not submitted. A contribution by J Carlsson-Ejdebäck et al. is included although it was not presented at the symposium. This inclusion is motivated by the relevance of the contribution to the subject discussed.

The symposium was held in association with the annual working meeting of the European Coronary Bypass Study Group. I am pleased to acknowledge that both the symposium and the meeting were generously supported by Boehringer Ingelheim. Desmond Julian and Edgar Sowton acted as chairmen of the symposium.

THE ANGIOGRAPHIC DEFINITION OF CRITICAL CORONARY STENOSIS

C. Richard Conti, M.D.,
Carl J. Peplow, M.D.,
Robert L. Feldman, M.D.,
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6 NIH Research Grant No. NIH HL-17717

Dr. Feldman is a Research Fellow of the Florida Heart Association

coronary blood flow (electromagnetic transducer) were measured at rest and following a 10 second coronary artery occlusion.

The upper left panel represents resting \downarrow reactive hyperemic coronary blood flow in a non-obstructive coronary artery. There is pressure gradient from aorta to distal coronary artery. Following a 10 second occlusion there is a small pressure differential and typical hyperemic response. The upper panel illustrates the effect of a 60 % lumen diameter reduction produced by a snare occlusion. There is no difference between resting coronary blood flow and is no resting pressure gradient. Following a 10 second coronary occlusion there is a \downarrow in the peak reactive hyperemic response and a clear cut pressure differential aorta to distal coronary artery. In the lower left panel the effect of an 80 % narrowing is represented. There was no resting aorta-coronary gradient nor was there a decrease in resting coronary blood flow following a 10 second occlusion there is marked decrease in the peak hyperemic response and a large pressure differential between aorta and distal coronary artery. In the lower panel a 90 % snare narrowing produces a marked decrease in resting coronary blood flow and a resting aorta coronary pressure gradient. Following 10 second coronary artery occlusion reactive hyperemia is essentially obliterated and the aorta-coronary pressure gradient is increased.

Since a 60 % lumen diameter reduction produced by a snare, had little effect on resting coronary blood flow and began to diminish the peak reactive hyperemic response to a second occlusion, we thought it appropriate

to evaluate the effect of lengthening the 60 % narrowings on coronary blood flow. Figure 3 illustrates the effect of lengthening a 60 % lumen diameter reduction on resting coronary blood flow, distal coronary artery pressure and the peak reactive hyperemic response. The upper left panel shows the effect of a 1 mm long 60 % narrowing. There was no difference between the narrowing and the 60 % snare occlusion shown in Figure 2. Upper right panel increasing the length of this 60 % narrowing to 5 mm, produces a striking increase in the resting aorta coronary pressure gradient and a slight decrease in resting coronary blood flow. The peak reactive hyperemic response is markedly decreased. Lower left panel increasing the length of the 60 % narrowing to 10 mm decreases the resting coronary blood flow and essentially obliterates the reactive hyperemic response. (Comparable to a 90 % lumen diameter reduction produced by a snare, shown in Figure 2.) Lower right panel the 60 % narrowing 15 mm long practically obliterates resting coronary blood flow and produces a marked pressure gradient. This occluder had to be removed quickly for obvious reasons.



Figure 4 Effect of percent narrowing and length of narrowing on resting coronary blood flow (see text)

Figure 4 summarizes the percent decrease in resting coronary blood flow in the group of eight animals studied. To the left of the figure are illustrated the effects of 70, 80 and 90 % narrowings produced by snare. It is obvious that a 90 % narrowing produced the greatest reduction in resting coronary blood flow whereas a 70 % narrowing had little effect. To the right of the figure are summarized data on the effect of increasing the length of 40-60 % coronary artery narrowings. It is obvious that

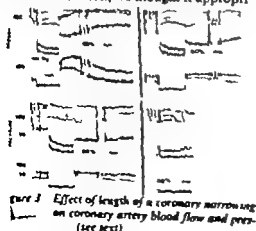


Figure 3 Effect of length of a coronary narrowing on coronary artery blood flow and pressure (see text)

The primary goal of coronary angiography is to identify sites of critical reduction in coronary artery lumen sufficient to produce myocardial ischemia. The human angiographer is faced with the problem "what is the physiologic significance of a coronary artery narrowing observed during angiography?" Gould et al and Lawrie et al have shown that a reduction in lumen diameter of 80 % or greater will reduce resting coronary blood flow in the laboratory animal (1,2). These observations have been extended to the human situation and have led to the view that an 80 % reduction in lumen diameter is sufficient to result in myocardial ischemia in the resting heart and certainly in the stressed heart.

Clinical observations in patients suggest that a coronary artery diameter reduction of less than 80 % may be sufficient to result in myocardial ischemia, particularly during periods of stress. Experimental studies in an animal model by Gould and Lipscomb (3) and Hillis and Freisinger (4) support the view that a 50 % reduction in lumen diameter may be sufficient to reduce coronary blood flow particularly during periods of increased demands.

Experiments in our laboratory confirm and extend the observations of Gould and Freisinger. In this communication we want to make three major points by developing three separate but related experiments. First coronary artery blood flow studies in an animal model to show that a long coronary artery narrowing or sequential coronary narrowing, affect resting and peak hyperemic coronary blood flow more than short or single narrowings. Second angiographic studies of coronary artery narrowings of known length and percent lumen diameter reduction in an animal model to show that coronary angiography is most accurate when the coronary artery narrowing is trivial or severe greater than 5 mm in length or when the narrowing is measured with calipers. Third, angiographic studies of the effect of nitroglycerin on percent lumen diameter reduction in man to show that nitroglycerin may alter the angiographic estimation of percent coronary lumen diameter reduction.

I CORONARY ARTERY BLOOD FLOW STUDIES IN AN ANIMAL MODEL

(A) The effect of the Percent Narrowing Length of Coronary Artery Narrowing on Resting and Reactive Hyperemic Coronary Blood Flow

Coronary narrowings were created in anesthetized open-chest dogs using a calibrated snare or Milled plastic occluders. Aortic and distal coronary pressures constantly monitored. Figure 1 is a left anterior oblique projection of a left coronary artery.

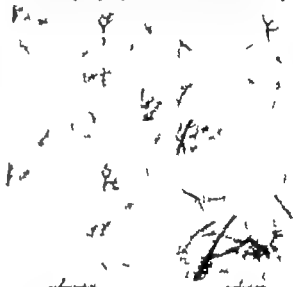


Figure 1 Angiographic appearance of narrowings created by Milled plastic occluder (text)

Figure 2 illustrates the angiographic appearance of a 60 % lumen diameter reduction produced by a 5, 10 and 15 mm long Milled plastic occluder.

A typical experiment in a single animal study is illustrated in Figure 2. In this experiment aortic and distal coronary pressures

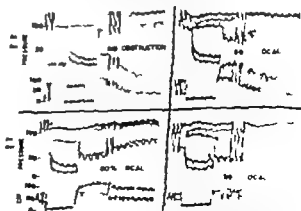


Figure 2 Effect of sharp coronary narrowings on coronary artery blood flow and pressure (see text)

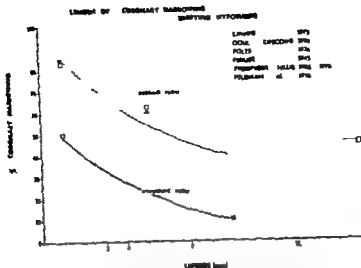


Figure 7 Comparison of different studies on the effect of length on resting and reactive hyperemic coronary blood flow (see text)

Conclusion

data indicate the following. First, short of 40–60 % do not decrease rest coronary blood flow but slightly decrease hyperemic coronary blood flow. Second, increasing the length of these 40–60 % from 1 to 5 mm further decreases peak hyperemic coronary blood flow. Third, increasing the length of 40–60 % narrowings to 10 mm decreases resting coronary blood and obliterates reactive coronary blood flow. Fourth, several narrowings of 40–60 % decreased peak hyperemic coronary blood flow greater than single narrowings of the same total

coronary arteries of animals, with known degree of narrowing and known length of narrowing were evaluated by two independent angiographers.

(A) Subjective and Objective Assessment of Coronary Angiograms

Coronary angiograms of 50 coronary artery narrowings were evaluated by two expe-

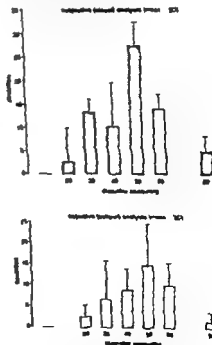


Figure 8 Accuracy of subjective and objective analysis of coronary angiograms (see text)

PROBLEMS ASSOCIATED WITH ANGIOGRAPHIC ASSESSMENT OF ARTERY NARROWINGS OF UNKNOWN LENGTH AND PERCENT DIAMETER REDUCTION

ly in the course of our animal experience, noted that the angiographic assessment of short narrowing (of proven severity) was usually interpreted as a much higher degree narrowing. For example, a 60 % narrow 1 mm in length appeared to be an 80–90 % coronary artery narrowing. As a result of these causal observations, an experiment as designed in which angiograms of the co-

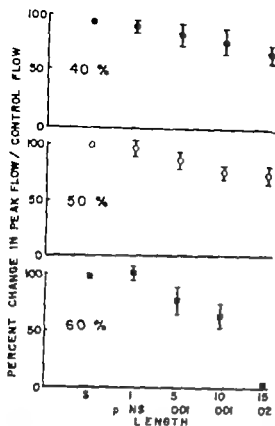


Figure 5 Effect of percent narrowing and length of narrowing on peak reactive hyperemia (see text)

as the length of the coronary narrowing increases resting coronary blood flow decreases. Similar results were obtained during reactive hyperemia as illustrated in Figure 5. In this illustration peak hyperemic flow is divided by control flow in order to normalize the response for each individual animal. The top panel illustrates the effects of a 40 % narrowing. As the points go from left to right the length of the coronary narrowing increases from the snare narrowing to a 15 mm long narrowing. It is obvious that as the length of the narrowing increases the peak reactive hyperemic response decreases in the 40, 50 and 60 % narrowings. The effect is most striking with 60 % narrowings.

(B) The Effect of Sequential Coronary Artery Narrowings on Resting and Peak Reactive Hyperemic Coronary Blood Flow

Preliminary experiments have been performed in which we compared the effect of a 60 % narrowing 2 mm in length to a series of two 60 % narrowings 1 mm in length. Figure 5 illustrates this comparison in a single animal study. The left panel shows no resting aorta-coronary pressure gradient and normal resting coronary blood flow. Following a 10

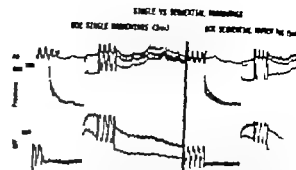


Figure 6 Effect of sequential coronary artery narrowing on resting and peak reactive hyperemic coronary blood flow (see text)

second occlusion a slight aorta-coronary pressure gradient develops and there is a typical reactive hyperemic response. The right panel shows an unchanged resting coronary blood flow but a slight resting aorta-coronary pressure gradient. Following a 10 second occlusion there is a clear cut aorta-coronary pressure gradient and a slight decrease of peak reactive hyperemic response. We have found this observation to be consistent in a small number of animals that have been studied. The explanation for this effect is not clear at the moment.

(C) Summary

Figure 7 summarizes our own data and compares it to that of others (5, 6, 7). The area contains data obtained during reactive hyperemia. Vertical axis represents the degree of coronary narrowing that was required to decrease coronary blood flow from control. Horizontal axis represents the length of the coronary narrowing. The dotted line represents the best fit curve for our data. The dashed line represents the best fit curve for resting coronary blood flow published by others. The solid line in the hatched area is the best fit curve for all data during reactive hyperemia. The data indicate that as the length of the coronary narrowing increases the percent narrowing required to reduce resting coronary blood flow decreases. For example, an 85 % narrowing, 1 mm in length will reduce resting coronary blood flow whereas a 55 % narrowing requires a length of 10 mm to have the same effect on resting coronary blood flow. The hatched area contains similar data during hyperemic coronary blood flow and again illustrates the striking effect of increasing the length of the narrowing on the reactive hyperemic response.

70% NARROWING

1mm long

5mm long

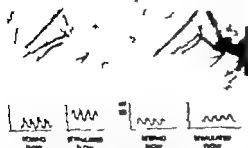


Fig 12 Coronary angiograms of a 70% narrowing 1 mm and 5 mm in length (see text)

panel) and 5 mm long (right panel). Nor in the 1 mm long narrowing and there is slight alteration of resting blood flow and a decrease in peak stimulated blood flow in 5 mm long narrowing. Figure 12 strikingly illustrates the discrepancy between the perception of the degree of narrowing, the actual degree of narrowing, measured postmortem in an artery with a 70% narrowing of 1 mm length (left panel) and 5 mm length (right panel). Resting and peak stimulated flows are slightly decreased in the long narrowing and markedly diminished in the 5 mm narrowing. Note the immediate appearance of collateral blood flow in the left anterior descending coronary artery, to the marginal branches of the circumflex in the right panel, at the arrow. Immediate appearance of collateral blood flow to distal marginal branch emphasizes, in this case, the physiologic abnormality produced by the longer narrowing.

(C) Conclusion

The foregoing observations relating to observer accuracy with coronary angiography can be summarized as follows. Errors are greatest with subjective estimates of coronary artery narrowings of less than 5 mm in length and with subjective estimates of coronary artery narrowings. Errors are least with objective measurements of coronary artery narrowings, narrowings measured with callipers.

III. PROBLEMS ASSOCIATED WITH THE USE OF SUBLINGUAL NITROGLYCERIN PRIOR TO CORONARY ANGIOGRAPHY. CORONARY ANGIOGRAPHIC EFFECTS OF NITROGLYCERIN ON PERCENT CORONARY LUMEN DIAMETER REDUCTION IN MAN

Nitroglycerin is commonly used in the angiographic laboratory in order to enhance visualization of coronary arteries. Often, angiograms are not performed prior to nitroglycerin administration. We initiated a study to determine whether or not the use of nitroglycerin affected the estimation of percent lumen diameter reduction in man.

(A) Study Design

We approached this problem in the following manner. Forty seven patients undergoing routine angiography had nitrates withheld for greater than 2 hours before angiography. Cine frames were matched to the same portion and part of the cardiac cycle pre nitroglycerin and post-nitroglycerin. The diameter of the projected coronary arteries and narrowings were measured with callipers to help reduce the error in interpretation. The percent coronary artery narrowing was determined by dividing the diameter of the narrowing by the vessel diameter immediately proximal to the narrowing and multiplying by 100.

(B) Results

Figure 13 shows the changes in coronary artery diameter following the administration of sublingual nitroglycerin. Each data point

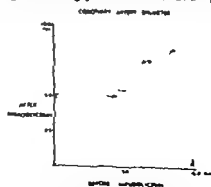


Figure 13 Changes in coronary artery diameter following the administration of sublingual nitroglycerin (see text)

perienced coronary angiographers without knowledge of the other's findings (8). Figure 8 illustrates the accuracy of our angiographic interpretation when compared to the true narrowing, (measured by coronary artery casts). The vertical axis is percent deviation from the true narrowing and the horizontal axis is the true percent diameter reduction. There was little error when 20 and 90 % narrowings were estimated subjectively. However in the arteries with 50 and 60 % lumen diameter reduction our error was much greater. The error consistently over-estimated the degree of narrowing. The lower panel summarizes the analysis of the same coronary arteries when measured with calipers. Once again the 20 and 90 % narrowings are close to the actual degree of narrowing whereas 50 and 60 % narrowings produced a higher degree of deviation from the actual value.

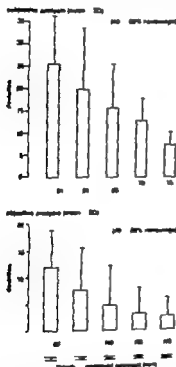


Figure 9 Accuracy of subjective and objective analysis of 40-60 % coronary artery narrowings (see text)

Figure 9 illustrates the accuracy of subjective assessment (top panel) of 40-60 % narrowings of varying length. It is visually obvious that as the length of the narrowing increases, the percent deviation from the true value decreases. Thus a 40-60 % narrowing of 1 mm in length was assessed least accurately. The lower panel is a similar analysis using calipers to measure the percent narrow-

ing. Clearly accuracy improves as length narrowing increases. We were most accurate for 40-60 % narrowings of 1 mm length, regardless of the method used.

(B) Relationship of the Visual 4 Percent Coronary Narrowing True Narrowing and Measured Coronary Flow

Figure 10 is a left anterior oblique left coronary angiogram without hyperemic



Figure 10 Coronary angiogram without hyperemia to illustrate resting coronary flow and blood flow after injection of angiographic contrast material

ary blood flow. Figure 11 illustrates a point about the visual over-estimation of coronary artery narrowings. This cine frame represents a true 50 % narrowing, 1 mm long

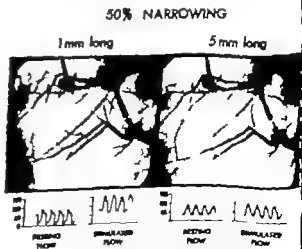
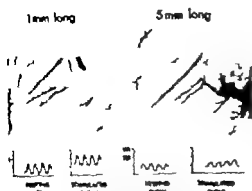


Figure 11 Coronary angiogram of a 50 % narrowing 1 mm and 5 mm in length (see text)

70% NARROWING



12 Coronary angiogram of a 70% narrowing 1 mm and 5 mm in length (see text)

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Conclusion

From the foregoing observations relating to observations with coronary angiography can be drawn as follows. Errors are greatest with narrowings of 40–60% lumen diameter reduction narrowings of less than 5 mm in length and with subjective estimates of coronary narrowings. Errors are least with total or severe narrowings, narrowings greater than 5 mm in length and narrowings reduced with calipers.

III. PROBLEMS ASSOCIATED WITH THE USE OF SUBLINGUAL NITROGLYCERIN PRIOR TO CORONARY ANGIOGRAPHY. CORONARY ANGIOGRAPHIC EFFECTS OF NITROGLYCERIN ON PERCENT CORONARY LUMEN DIAMETER REDUCTION IN MAN

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(B) Results

Figure 13 shows the changes in coronary artery diameter following the administration of sublingual nitroglycerin. Each data point

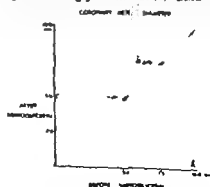


Figure 13 Changes in coronary artery diameter following the administration of sublingual nitroglycerin (see text)

represents a pre and post-operative coronary diameter. Vessels that failed to increase in size following sublingual nitroglycerin fall on the line of identity. Figure 14 shows the change in the diameter of the narrowing. The data illustrate that the majority of coronary artery narrowings do not change diameter



Figure 14 Changes in the diameter of the coronary narrowing following the administration of sublingual nitroglycerin (see text)

following nitroglycerin administration. However, of those that did change diameter, the majority increased in size. Figure 15 illustrates the change in percent coronary narrowing after nitroglycerin administration. Obviously, the response is variable but the majority of patients increased the percent of coronary narrowing following nitroglycerin. The shaded area, is of particular interest since these data points represent vessels narrowed less than 50% prior to nitroglycerin. Following the administration of nitroglycerin the percent narrowing increased to greater than 50%.

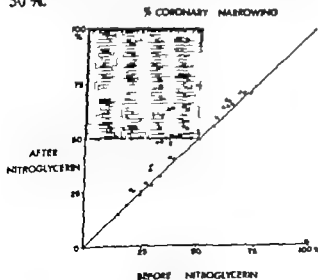


Figure 15 Change in percent coronary narrowing after sublingual nitroglycerin (see text)

(C) Conclusion

Our observations indicate that administration of sublingual nitroglycerin dilates the coronary artery proximal to narrowing, whereas coronary arteries usually remain fixed after nitroglycerin. Therefore, the percent narrowing usually increases. However, this is not universally the case and the variability of the response must be considered when evaluating the narrowing of individual coronary arteries. It is obvious that nitroglycerin may influence the interpretation of coronary

IV SUMMARY

It is hazardous to rely solely on angiography to define critical coronary artery stenosis. The physiologic significance of a narrowing observed at coronary angiography can best be evaluated by the addition of stress testing, i.e. exercise or pacing and cardiac catheterization with ECG monitoring, as well as resting and exercise isotope perfusion studies. However, when evaluating coronary angiograms for critical coronary artery stenosis, several points are worth considering. First, a long narrowing will decrease coronary blood flow more than a short narrowing of the same severity. Second, sequential narrowings will decrease coronary blood flow more than a single narrowing of the same total length. Third, a long narrowing can be estimated more accurately than a short narrowing. Fourth, caliper measurements of coronary artery narrowings are more accurate than subjective estimation. Fifth, coronary artery dilators may increase the percentage narrowing in some cases.

The complex effects of percent stenosis and length of stenosis on the physiology of coronary blood flow must always be considered when evaluating angiograms. In addition, the angiographic or photographic artifacts produced by short narrowings tend to over-estimate the percent stenosis and the calipers should be used to measure percent stenosis especially in the short narrowings. Finally, nitroglycerin should not be used routinely prior to coronary angiography.

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3.

ORONARY ARTERIOGRAPHY—AN OBJECTIVE METHOD?

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Coronary arteriography is considered necessary today for decisions on treatment and management of patients with coronary artery disease. The coronary arteriogram is in fact the basis on which most of these decisions are made. (1)

The purpose of performing coronary arteriography is to detect and grade coronary artery stenosis.

The value of coronary arteriography is based on the assumption that coronary artery flow is inversely related to the degree of stenosis as we see them on the coronary arteriograms. The further assumption is made that this flow is related to the nutrient flow or to the demands for nutrient and oxygen in the muscle in an area distal to the coronary artery stenosis. The visualization and evaluation of collaterals are based on the same assumption.

Obviously these assumptions can be and have been questioned. Let us avoid this problem and assume that there is a relationship between the flow in the coronary arteries and the stenosis as we see them on the coronary arteriogram.

It is highly desirable that the coronary arteriograms are technically satisfactory. The chances of detecting and grading stenosis decreases with poor image quality.

There is a quality program with coronary arteriography and suboptimal coronary arteriograms are performed every day. Does this mean that some patients are not receiving adequate medical care because of poor quality coronary arteriograms? I would say no and suggest that this means that high quality coronary arteriography is *not* necessary for good medical care of patients with coronary artery disease.

But this is not the problem to be discussed here and let us assume that all coronary arteriograms are of optimal quality.

The next question will then be: what is the optimal quality of coronary arteriograms? Most radiologists, cardiologists and laymen prefer high contrast black and white arteriography. They do so regardless of the information content of the arteriogram. This is an interesting psychological phenomenon. I am not qualified to go into that and I will just make a few remarks. One reason for preferring the high contrast black and white arteriograms is that you can make excellent slides and illustrations from them.

The psychological impact of the high contrast high resolution arteriogram is able and the emotional effect of it has been realized and used.

I would like to submit that the high contrast arteriogram is not necessarily the best arteriogram containing the most valuable information. But this is again not the point to be discussed, so let us assume that we have the best coronary arteriograms available.

Then, how do we assess our coronary arteriogram? We look at them and what angiographers do is to express a stenosis as a percent reduction of the coronary artery compared to the pre-stenosis size of the artery.

All reports concerning treatment and prognosis of coronary disease have tables listing the stenosis of the coronary arteries as 30, 50, 90, 99 percent, etc.

Percent reduction of what? Cross sectional area or even circumference? If you ask this question angiographers usually become puzzled and after a while most will say that they mean reduction of cross sectional area.

This answer implies that in fact, we have a considerable subjective element in the assessment of coronary artery stenosis.

For what we can observe directly on a two-dimensional coronary arteriogram are only the diameters in different segments of the artery.

If we are to arrive at a percentage reduction of cross sectional area after observing diameters, we have to perform calculations that are impossible for most individuals without aid of a calculator or pen and paper.

This means that when angiographers are talking about percent stenosis referring to cross sectional area, they are not very objective but using an impression, or if you will, a gestalt approach to the problem. In other words, they have a "feeling" for what a 70% stenosis should look like.

The impression or interpretation of coronary arteriograms varies also from individual to individual and as travelling angiographers you can easily make the observation that 50% stenosis in Europe, for instance, is not the same as 50% of stenosis in the U.S.A.

So we have circumstantial evidence that there are considerable subjective elements in assessing coronary artery stenosis.

to investigate this in more detail a study recently performed. (2) The purpose of study was twofold.

To study observer performance in assessing coronary artery stenosis.
Compare the diagnostic information on 16 mm cine film and 70 mm fluorography

JDS

30 unselected patients with CAD
Judkin's technique for coronary arteriography

Both 16 mm cine and 70 mm camera recordings of 42 projections of left and 32 projections of right coronary artery
The same T¹²⁵ cesium-iodide image intensifier and 0.6 mm focal spot tube for both recordings.

16 mm cine at 50 frames per second
70-100 Kv and 8 mR per frame.

70 mm films at 4 frames per second 70-100 kV and 80 mR per frame.

Dose of contrast agent and rate of injection constant.

16 mm cine and 70 mm film viewed independently in random order by four trained observers without knowledge of the patient.

Each recording studied separately location of stenosis, degree of stenosis (% reduction in diameter) and presence of collateral circulation recorded.

Two months interval between viewing 16 mm cine and 70 mm films and between first and second viewing.

In addition 29 stenoses measured on 70 mm films within 0.1 mm

JLTS

There was no difference between these observers' ability to detect stenosis when using 16 mm cine film and 70 mm films.

There was considerable inter-observer difference in estimating the degree of coronary artery stenosis (fig. 1). This was true for both mm cine and 70 mm film observations. In instances the observers' estimates were at variance with the estimates of coronary artery stenosis in the official report of the department of radiology

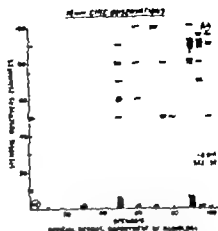


Fig. 1 Comparison between estimates of coronary arterial stenosis in the official report and by four independent observers using 16 mm cine film. Figures in circles indicate report-observer agreement on 0 percent stenosis (46) and on 100 percent stenosis (63). Similar results were obtained with 70 mm films

2. There were also considerable intra-observer differences in separate observations of 16 mm cine films and 70 mm films (fig. 2).

In 18 % of the cases the observers changed their estimate across the 50 % diameter stenosis line between the two observations.

3. There was nearly complete agreement regarding the presence and location of collaterals with both 16 mm and 70 mm films.

INTRA-OBSERVER DIFFERENCES ON 70mm FILMS

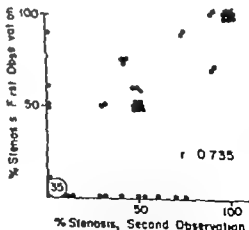


Fig. 2 Intra-observer differences in separate observations on 70 mm films. Each of the four observers reviewed the films twice with a 2 month interval between readings.

The following additional observations were made

- Among the four observers no two agreed on a definitely erroneous interpretation
- All observers had variations of estimates in both directions.
- The widest variation between estimates were found with stenosis in LAD
- Observer performance improved only little with longer experience.
- Complete agreement as to the location of stenosis when recorded

One way to improve the accuracy of coronary arteriography assessment is to make measurements on the coronary arteriograms. Several investigators have tried this and expressed varying degrees of confidence in their results. Others have been critical about the possibilities of measuring small vessels in patients

Since there seems to be some confusion regarding the possibilities of *actual measuring coronary artery size on cine films or 70 mm films* some simple experiments were performed using plastic tubes as models for coronary arteries and the best imaging systems available at present. (3)

The same recording systems were used as in the study on observer performance. The conditions for imaging were optimum and different from conditions present in patients in many respects. The degree of magnification could be calculated exactly. The tubes were curved only in one place. The object did not move and the tube was constantly filled with undiluted contrast medium. The angiographic measurements of cross sectional areas were compared with the true cross sectional areas of the stenosis on the tube. Generally the angiographic measurements were not very accurate (Fig. 3). As expected the incidence of the central beam had great influence on the accuracy of the measurements.

It appears that true measurements of coronary artery stenosis and of coronary arteries with diameters below 3 mm simply are not possible in patients even with the best recording systems commercially available. This is not totally expected but it is important to emphasize this limitation of the present imaging systems. These findings partly explain the difficulty in estimating physiologic effects of anatomic lesions as they appear at coronary angiography

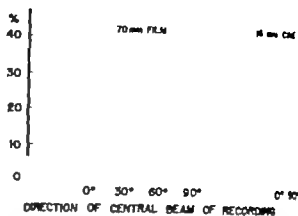


Fig 3 Accuracy of angiographic measurements of stenosis of a plastic tube. The deviation from the true cross sectional area is given in percent

SUMMARY AND CONCLUSION

There is considerable inter and intra observer error in estimating coronary artery stenosis on coronary arteriograms

There is no obvious way to increase the accuracy of coronary arteriography

Measurements are of little or no value in arteries and structures below 3 mm in size.

Coronary arteriography is only a semiquantitative method for making decisions on treatment prognosis and follow up of patients with coronary artery disease

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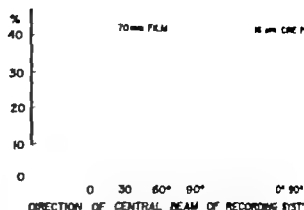


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4.

COMPUTER GENERATED INDEX FOR THE ASSESSMENT OF CORONARY ANGIOGRAPHY

R. Balcon
M.R. Catzall
D.L. Stone,
G. Fierlicht



Calculation of the index

$$Wi = 1 - \frac{Li}{4}$$

Wi is the weighting of the *i*th vessel
Li is the size of the lesion in the *i*th vessel and its value varies from 0 to 4 where

- 0 = No lesion
- 1 = 25 % lesion
- 2 = 50 % lesion
- 3 = 75 % lesion
- 4 = 100 % lesion

al coronary index is calculated according to the formula

$$= \frac{100}{NTV}$$

$$1[W2 + W2W3(W4 + W5) + W6(W7 + W9)] + W10(W11 + W12 + W13)$$

where NTV = number of terminal vessels.

This system yields values for the indices between 0 and 100 where 0 corresponds to obstruction and 100 to no obstruction. Retrograde filling as seen in the angiogram can be incorporated into the model. 2 levels were applied, 50 % and 100 %.

To reduce the relevant lesions to half and to their original weighting respectively. Data used in this study originated from the angiograms of patients with suspected coronary artery disease. At present the data it contains information on more than 100 patients.

RESULTS AND CONCLUSIONS

Angina grade

Angina was classified according to NHA from 0 (no angina) to 4 (rest pain). Figure 2 shows the relationship of the index to angina grade. The average value of the index for each of the grades of angina was calculated and plotted. Good relationship exists for grades 0-3. Low values of the index are associated with high angina grades i.e. severe angina.

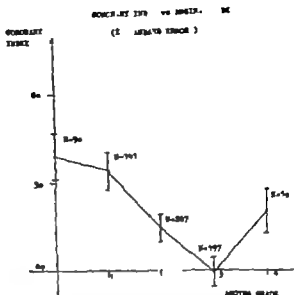


Figure 2

The mean value of the index rises for patients with rest pain.

Mean value of coronary index for patients with angina grade 1 or less was significantly different from the rest of the group. The patients with grade 2, 3 and 4 angina had a lower average CI (42.4) compared with the grade 1 patients (51.5 $p = 0.0001$ $n = 523$).

In addition Fig. 3 shows that the shape of the curve is unaffected by 50 % or 100 % corrections for retrograde filling. The rise in index for patients with rest pain is probably explained by the heterogeneity of that group of patients which may even contain patients with normal coronary arteries or only minor disease.

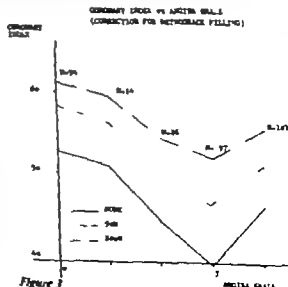


Figure 3

ABSTRACT

Mortality in patients with coronary artery disease is related to its severity. The commonly used classification of 1, 2 or 3 vessel disease is relatively insensitive. We have designed a new classification which takes into account site, severity and effect of multiple lesions in the coronary circulation. Data is recorded on Mark Sense computer cards and a coronary index (CI) obtained. We have collected data from 1100 patients and shown correlations of the index with clinical variables, ventricular function and in particular mortality.

The prognosis of an individual patient with ischaemic heart disease is related to pathology documented by coronary arteriography. Survival is a function of the number of vessels involved and the severity of the obstructions.¹⁻³ The natural history of patients with stable angina has been reported by several centres.⁴⁻⁸ Those retrospective studies used varying criteria for selection and were performed before the introduction of bypass surgery. In 1974 Reeves⁹ published his analysis of existing data which suggested an annual mortality for single vessel disease was 2.2 %, for double 6.8 % and triple 11.4 %, with five year mortalities of approximately 17 %, 38 % and 55 % respectively. These studies encompass the whole spectrum of ventricular function and this emerges as the second major factor in prediction of survival. Several investigators have shown that with poor left ventricular function (abnormal haemodynamics, reduced ejection fraction and wall motion abnormalities) five year survival is considerably reduced.^{4,5} Aortocoronary bypass grafting relieves angina in 80-90 % of patients.¹¹⁻¹⁵ There is some evidence to suggest it improves ventricular function.^{16,17} The controversy regarding its possible beneficial effect on life expectancy persists.²¹⁻²⁵

Many centres now have very low operative mortality (0.7-1.5 %)^{23, 24, 25} and impressive (93-98 %) three year survival curves, when compared with the natural history data.²³⁻²⁷ Patients are however selected and usually excluded with either diffuse coronary disease or poor left ventricular function. Other reports suggest that survival is only improved in the patients with left main stem stenosis.^{18, 20, 21, 22} In particular the only randomised prospec-

tive study involving all groups of patients with coronary disease.¹⁸ The high risk of left main stem lesion is related to its proximity prejudicing the blood supply to the left ventricular muscle. Friesinger et al. also pointed out the importance of site stating that survival of patients with lesions of anterior descending coronary artery depended upon whether it was proximal or distal to the first septal branch. They also related the severity of lesions from 0-5. We have developed a scoring system^{28, 29} that takes account the severity of lesions on a 6 point scale, their site, the effect of multiple lesions and possible effects of retrograde filling. Data is collected on computer Mark Sense cards so that multiple methods of analysis can be assessed.

METHODS

Definition of coronary index

The definition of the index is based on assumptions about the nature of the relationship between the lesions in various branches of the coronary arteries and the magnitude of their overall effect.

1. Serial lesions multiply in effect.
2. Parallel lesions add in effect.
3. The effect of a lesion in any given vessel is directly proportional to the degree of encroachment on arterial diameter.

The proposed weighting system consists of weights W1-W13 corresponding to vessels V1-V13 (Fig. 1). The weights multiply add according to the positions of the lesions to form the final value of the index.



Figure 1

or all three of the vessel territories. The of one second generation lesion or without lesions in the third generation u lity to 8 % per annum, and or three second generation lesions to 2 (Fig. 6)

ortality correlates reasonably well with Indices <20 are associated with high lity rates (12-26 %) which fall with asing coronary indices. (Fig. 7).

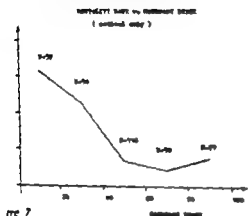
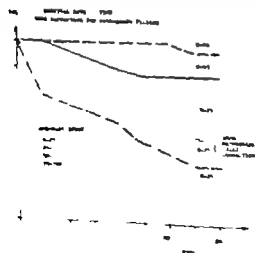


Fig. 8 shows the survival curves for the indices of coronary index. The mortality for patients with indices 50-100 is very low and numbers small. With increasing severity of disease however survival becomes progressively worse. Correction for retrograde flow has little effect on the survival curves in particular does not improve the prog



nosis of patients with 0-50 indices with severe disease.

This method of analysis therefore seems to provide a reasonably sensitive means of defining high risk groups and potentially a method of comparing various forms of therapy

Exercise load

Exercise tolerance was assessed using bicycle ergometry. This was measured in increments of 150 kpm per minute starting at 150 kpm per minute. Maximum achieved load was measured.

Patients who achieved maximum exercise levels <450 kpm/min had a significantly lower CI than those able to reach higher work loads (42.3 cf 49.7 $p = 0.0001$ $n=472$) Fig. 4. Retrograde filling has been evaluated by assuming 50 or 100% effectiveness. This only elevates the curves but does not change their shape suggesting that its presence does not affect exercise tolerance.

The group of patients unable to achieve 450 kpm/min during bicycle ergometry has

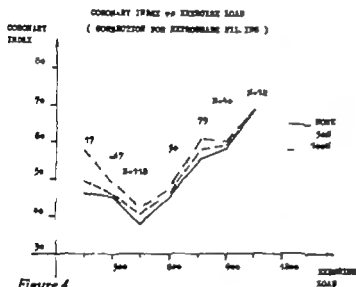


Figure 4

a higher mean value of index than might be expected. We think that this is due to the inability of certain patients to ride the bicycle and who are therefore unable to do exercise properly. It is important to note that the test was not a diagnostic exercise test, but one to assess maximal exercise capacity for follow up purposes and that angina was therefore not necessarily the end point of the investigation.

Left ventricular contraction

Figure 5 shows the relationship of the index to LV contraction when assessed by number of areas of akinesia or dyskinesia. Low values of the index are associated with more exten-

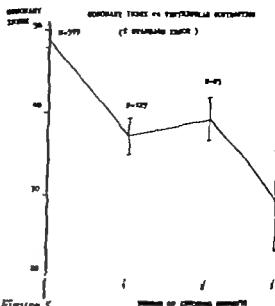


Figure 5

sive contraction abnormalities. If left ventricular contraction is defined as 0 or more areas of akinesia on the angiogram, there is a significant difference between values of the index for normal and abnormal ventricles (45.3 cf 39.2 $P = 0.05$ $n = 802$).

Mortality

The value of the index as an indicator of prognosis will depend on its direct relationship to mortality. In this section only patients treated medically are considered.

The site of severe lesions (grade 3 & 4) first evaluated in 297 patients treated medically and followed for up to two years. Patients with third generation lesions i.e. in terminal branches only had an annual mortality rate of 2% despite the fact that approximately 40% of these patients have lesions

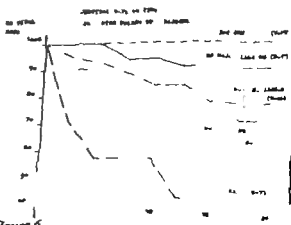


Figure 6

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**SUDDEN OUT-OF HOSPITAL CORONARY DEATH AND
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Efforts to control coronary heart disease (CHD) in the population have a close relationship to prevention of sudden death as more than half of CHD patients will die suddenly.¹⁻⁴ In Kaunas, out-of-hospital mortality due to acute CHD in 1969 accounted for 67.8% of all fatal cases of definite and possible myocardial infarction (MI). Special efforts to improve the organization of prehospital medical care resulted in the decrease of out-of-hospital mortality to 47.4% (Table 1) in 1976. Owing to the lack of clear-cut manifestations of cardiovascular disorders in around one fourth of CHD patients most of the deaths were unexpected occurring too rapidly for the persons to arrive at the hospital before death.⁹⁻¹⁰

According to current views the problem of prevention of sudden out-of-hospital coronary death is closely related to the prevention and treatment of coronary atherosclerosis. Chronic obstruction of 75% or more in one of the major subepicardial coronary arteries at least is noted by many observers in the majority of sudden death cases.^{2, 11-19}

The fact that coronary atherosclerosis is a progressive disease which is manifest episodically²⁰ is confirmed by current angiographic studies of the natural history of coronary artery stenosis.²¹ Anatomical characteristics of atherosclerotic changes in coronary arteries and myocardium are to our mind of particular interest when obtained from the representative population contingent

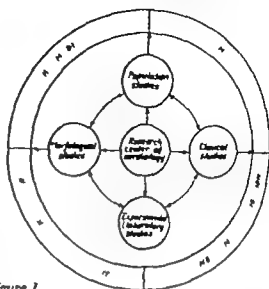


Figure 1

METHODS AND MATERIAL

In Kaunas Research Institute of Cardiology extensive studies covering various aspects of CHD control in the population are being carried out (Fig. 1). Population clinical, epidemiologic, and experimental investigations are conducted making up an integrated system permitting analysis of the various aspects of healthy population, touching all the aspects of the natural history of CHD. All these investigations are performed in close cooperation with the existing health delivery system — inpatient and outpatient medical services and public health authorities.

Table 1
Myocardial infarction register data for Kaunas

Year	Total of MI recorded	MI incidence per 1 000	Deaths			Mortality in hospital %	Death/percentage/total	Proportion of sudden in % of the total death
			Total	In hospital	Outside hospital			
1970	328	1.06	154	44	110	19.8	46.9	71.4
1971	388	1.27	160	44	116	16.2	41.2	72.5
1972	427	1.39	188	66	122	21.6	44.0	64.9
1973	447	1.46	177	74	103	20.5	39.6	58.2
1974	489	1.45	193	76	117	19.6	39.5	60.6
1975	476	1.4	167	77	91	18.9	35.3	54.2
1976	501	1.4	167	77	82	20.4	34.5	54.2

this paper presents some data on sudden death cases accumulated in various of the population study (Fig. 2). Given covers the population group at high risk, i.e., the male population of middle It is analysed in the following way: the Kaunas male population aged 45-65; then separately one of its segments (%) representing the cohort of multifactorial CHD intervention study (this contingent been preliminarily examined and classified into risk groups). The study has started in 4 and the examined population was pro-

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tively followed up for five years.²² Material covering the whole Kaunas population without sex or age restrictions contributed to the identification of sudden coronary death cases and their selection for special morphologic study. Cases of sudden of hospital coronary death were identified by using diagnostic follow up data, reliable medical records and findings of special morphologic investigations. A case of coronary death was considered as such when it had occurred within 24 hours of the onset of terminal event irrespective of whether there was a past history of CHD or not, and if a complete autopsy examination ruled out noncoronary causes of death. The scope of the special morphologic study comprising 127 cases of sudden out-of-hospital death is shown in Table II. In all 127 cases, special morphologic investigations of major coronary arteries and myocardium were performed. In 88 cases, post mortem angiograms were made. Right and

Table II
Incidence of Autopsies, Special Morphologic and Angiographic Investigations of Coronary Arteries and Myocardium in Cases of Sudden Coronary Death among Kaunas Male Population Aged 45-65

Scope of investigations	Whole male population (1970-1975)		Contingent of prospective epidemiologic study (1972-1977)	
	No.	%	No.	%
Total number of sudden coronary death	193	100	25	100
Autopsy performed	165	85.5	23	92
Autopsy and special morphologic investigation	127	65.3	22	88
Autopsy special morphologic investigation and angiography	88	45.6	11	44
Autopsy not performed	28	14.5	2	8

left coronary arteries were filled with barium gelatine mixture under a pressure of 120 mm Hg using Schlenker apparatus for coronary injection. After angiography coronary arteries were examined by cross-sectioning at 1.5- to 2-mm intervals. The following points were determined: the type of coronary artery distribution, location, degree, extension and character of obstructive lesions and the presence of collateral formations. Special instrument was used to determine the degree and extension of obstructive lesions. Obstructions of 50% or more were taken into consideration. According to the extent, these obstructions were classified into short or local (less than 10 mm), tubular (5-15 mm), and diffuse (more than 15 mm or multiple local in the same segment). The mapping of all those pathologic changes were performed using the scheme recommended by the American Heart Association.²⁴ The chronic myocardial lesions were assessed by gross morphologic examination and histologic study of transverse slices of myocardium at intervals of 0.5-1 cm and by quantitative percentual evaluation of transmural scars.

RESULTS

Some clinical features obtained from the available medical records and diagnostic follow up in the 127 cases are presented in Table III

Table III

Characterization of 127 Cases of Sudden Coronary Death

History of Coronary Heart Disease	75 %
Hypertension	19 %
Diabetes mellitus	4 %
Length of terminal event less than 1 hour	46 %
Length of terminal event 1-6 hours	54 %
Average age of analysed cases	54 years

Chronic lesions of coronary arteries

Chronic obstructive lesions (atherosclerotic narrowing and old thrombotic occlusion) of 50 % or more have been found in all 127 cases. Those changes were present in at least one of four major coronary arteries (Table IV). In cases when significant obstruction was revealed in single or two arteries, luminal narrowing of lesser degree was found in other major coronary arteries as well in cases with luminal obstruction of 75 % or more, other arteries exhibited stenosis from 50 to 75 %, and in cases with narrowing of 50-75 % they exhibited 25 to 50 % resp. Chronic obstruction of 50-75 % was found in 10 % of cases and as a rule, two three or all four major coronary arteries were affected. Obstructive

Table IV

Number of Coronary Arteries with Chronic Obstruction in 127 Cases of Sudden Coronary Death

Number of affected arteries	Chronic obstruction (%)			
	50-75 %		75 % or more	
	No.	%	No.	%
One	1	1	48 (14 §)	38 (29 §)
Two	5	4	49 (22 §)	39 (45 §)
Three	4	3	12 (8 §)	9 (67 §)
Four	3	2	5 (2 §)	4 (40 §)
Total	13	10	114 (46 §)	90 (36 §)

§ — with old thrombotic occlusion

lesions of 75 % or more were present in of cases, most often in two or one major artery (39 % and 38 % resp.) and more in three or all four coronary arteries (13). Old thrombotic occlusions were present in 36 % of examined cases. The greatest number of old thrombotic occlusions was found in two or three arteries were involved. In 3 cases organized thrombi were located in both affected arteries. Recent thrombi were most frequent finding in those cases where stenosis of 75 % or more occurred in single two major coronary arteries. In 5 cases combined with old occlusions in other major coronary arteries. On the whole, the left anterior descending artery (Table V) was the most frequent site of chronic obstructive lesions.

Table V

Location of Chronic Obstruction of 50 % or More in Separate Coronary Arteries in 127 Cases of Sudden Coronary Death

Affected artery	%
Left anterior descending (LAD)	93 (9 §)
Right coronary artery (RCA)	76 (21 §)
Left circumflex (LC)	65 (7 §)
Left coronary artery (LCA)	22 (1 §)

§ — with old thrombotic occlusion

50 % or more, and the right coronary was more readily affected by old occlusions (21 %). When chronic obstruction both of 50-75 % and of 75 % or more, was present in two or three coronary arteries, most frequent combinations of involved arteries were RCA-LAD, LAD-LC and RCA-LAD-LC (Table VI). When a single artery was affected by obstruction of 75 % or more, it was RCA or LAD that was involved with equal frequency in such cases stenosis of lesser degree (50-75 %) also occurred in the other arteries in the same combinations mentioned above. Thus, the combinations of affected arteries, taking into consideration the lesser degree of obstruction as well were identical in these cases too.

Due to technical reasons angiograms of 76 cases only were analysed. In all 906 individual segments of major coronary arteries were evaluated (Table VII). Chronic obstruction of 50 % or more was found in 325 (36 %)

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 Distribution of Affected Coronary Arteries in
 Cases of Sudden Coronary Death

Affected Arteries	Chronic Obstruction (%)			
	50-75 %		75 % or more	
Combination	No.	%	No.	%
LCA	—	—	1	0,8
LAD	—	—	17	13,4
LC	1	0,8	9	7,2
RCA	—	—	21	16,6
RCA LAD	2	1,5	23	18,1
RCA LC	—	—	5	3,9
LAD-LC	2	1,5	19	15,0
LAD-LCA	1	0,8	2	1,5
RCA				
LAD-LC	3	2,3	10	8,0
LCA-RCA				
LAD	1	0,8	1	0,8
LCA-LC				
LAD	—	—	1	0,8
LCA LAD- LC RCA	3	2,3	5	3,9
All	13	10	114	90

them luminal narrowing of 50-75 % in 17 % (17 %), and that of 75 % or more in 17,2 % of the evaluated segments. The most frequent site of obstruction both of 50-75 % and of 75 % or more, was found to be the proximal and middle segments of all three coronary arteries and in the left main stem. In the left anterior descending and the right coronary arteries atherosclerotic changes of intimal and diffuse type (5 mm and more), of the complicated by total occlusion, were the main features. In the left circumflex artery and the left main stem, short local (less than 5 mm) and tubular and diffuse (more than 5 mm) obstructions occurred with all the same frequency. On the whole, in two thirds of all revealed pathologic changes consisted of obstructive lesions of more than 5 mm. In 36 cases of obstruction of 50 % or more and in 1 case of obstruction of 75 % intercoronary collateral filling was not found. It existed in almost all cases with 3 affected arteries, in two thirds of cases when 2 arteries were affected, and in one third of

Table VII
 Incidence of Chronic Obstruction in the Individual Segments of Major Coronary Arteries in 76 Cases of Sudden Coronary Death (Postmortem Angiographic Data)

Individual segments of coronary arteries	Incidence of obstruction (stenosis occlusion)		Chronic obstruction			
	Number of evaluated segments		Number of found lesions		50-75 %	75 % or more
	No.	%	No.	%	No.	%
RCA	1	72	30	41	13	18
RCA	2	69	33	48	15	22
RCA	3	59	20	33	9	16
RCA	4	56	4	7	1	2
LCA	5	45	10	22	3	7
LAD	6	71	58	82	36	51
LAD	7	75	46	61	19	25
LAD	8	69	23	33	14	20
LAD	9	68	22	32	12	18
LAD	10	46	13	28	5	11
LC	11	76	25	33	14	18
LC	12	74	12	16	5	7
LC	13	73	20	27	5	7
LC	14	35	8	23	2	6
LC	15	18	1	5	—	—
Total		906	325	36	153	17

According to AHA

cases when a single artery was damaged. The right type of coronary artery distribution predominated.

Heart Weight

Heart weight was increased in most cases and in 85 % was over 400 g (Table VIII). There was no direct correlation between heart weight and the number of affected arteries and the length of terminal event. The increase in heart weight was more evident in cases when old transmural MI extended to 30 % or more and particularly in patients with hypertension.

Old myocardial infarctions (scars)

These were found in 87 cases (68 %) of sudden coronary death (Table IX). Old transmural MI was present in 52 cases (41 %) and nontransmural ones in 35 (27 %). Moreover

Table VIII

Characterization of Heart Weight in 127 Cases of Sudden Coronary Death (In connection with Length of Terminal Event)

Length of terminal event	400 g		400—499 g		> 500—599 g		> 600 g		Total number	Average weight g
	No	%	No	%	No	%	No.	%		
1 hour	3	5	25	42	16	27	15	26	59	511
1—6 hours	16	24	28	41	18	26	8	9	68	467
Total	19	15	53	42	34	27	21	16	127	

in 5 cases of transmural scar and in 1 case of nontransmural scar they were paired. Transmural infarction was found more frequently in persons who had died within 1 hour than in those dying within 1—6 hours (51 % and 32 % resp.). Anterior and antero-septal and posterior and posteroseptal scars (transmural and nontransmural) were present with similar frequency. There was a tendency for more frequent occurrence of old transmural posterior and posteroseptal infarction in persons who died within 1 hour of the onset of terminal event. Old infarction was found more often when more than one artery was affected. However, when two, three and four coronary arteries were involved, the frequency of old infarction was similar (Table X).

COMMENT AND CONCLUSIONS

Chronic obstruction (atherosclerotic stenosis and old thrombotic occlusion) was present in

90 % of 127 examined cases of sudden coronary death. Atherosclerotic stenosis of 75 % or more was found in 10 % of cases, old thrombotic occlusion in 36 %.

Predominantly (in 77 %) one or two major coronary arteries had obstructive lesions of 75 % or more. Chronic obstructive injury, lesser degree (50—75 %) involved, as a rule two, three or all four major coronary arteries. However, when single or two arteries exhibited severe obstruction, other major arteries had luminal narrowing as well but of lesser degree. When luminal narrowing reached 75 % or more, stenosis of other arteries was found to be of 50—75 % and in the cases of narrowing of 50—75 %, 25—50 %. Typically, old thrombotic occlusion took place most frequently when two, three or all four major arteries were involved and was local in the right coronary artery predominant. The left anterior descending and right coronary arteries were the most common sites

Table IX

Characterization of frequency, extension and location of old myocardial infarction and the length of terminal event in 127 cases of sudden coronary death

Length of terminal event	No of cases	Total No of MI	Transmural								Not transmural								
			Anterior and antero-septal		Posterior and postero-septal		Both anterior and posterior		Total	Anterior and antero-septal		Posterior and postero-septal		Both anterior and posterior		Total			
No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%	No	%		
< 1 hr	59	45	35	12	20	16	27	2	4	30	51	10	17	3	5	1	2	14	24
1-6 hr	68	42	33	8	12	11	16	3	4	22	32	9	13	12	18	—	—	21	31
Total	127	87	68	20	16	27	21	5	4	52	41	19	15	15	12	1	0.8	35	27

Relationship between the Number of Affected Coronary Arteries (Obstruction of 50% or More) and Frequency and Extension of Old Myocardial Infarction in 127 Cases of Sudden Coronary Death

of	No. of cases	Old myocardial infarction					
		Total	Trans-mural	Non-trans-mural			
		No.	%	No.	%		
	49	57	14	29	14	29	
	54	41	76	28	5	13	24
	24	18	75	10	42	8	33
	127	87	68	52	41	35	27

lesions (93 % and 77 % resp.) such involving the proximal and middle of these arteries. Tubular and diffuse (length of 5 mm and more) types of stenosis predominated. When chronic obstruction was simultaneously present in several arteries, mostly it was a combination of left anterior descending and right coronary arteries.

Noteworthy is the fact that in the cases of obstruction of 75 % or more in a single artery it was mostly the right coronary or left anterior descending arteries that were affected.

Such injury was usually accompanied by stenosis of 50–75 % in the left anterior descending and circumflex branches and made the same combination of affected arteries as in cases when two arteries had luminal damage of 75 % or more. In view of the data suggesting that natural progression of stenotic coronary atherosclerosis is predominantly in the proximal and middle segments of left anterior descending and right coronary arteries,^{21, 22} the preliminary injury in only one of them, must alert

prospectively to the possibility of acute episodes of CHD. In our opinion, obstruction of 75 % or more in two coronary arteries in most cases is apparently the triggering situation in the development of transmural MI and death.

The results of our study allow us to surmise that sudden coronary death is an acute event in the history of chronically advancing athero-

sclerotic heart disease. These data may appear helpful for the more precise definition of criteria for the selection of candidates for coronary artery surgery.

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SUMMARY

Some data on sudden coronary death pathology from CHD population study in Kaunas are presented. 127 cases of sudden out-of-hospital coronary death were identified by means of diagnostic follow up data, available medical records and findings of special morphological investigations of coronary arteries and myocardium. In 76 cases of them postmortem angiograms were made. Chronic obstruction (atherosclerotic stenosis and old thrombotic occlusion) was present in all cases, obstruction of 75 % and more forming the majority (90 %) of cases. Old thrombotic occlusion occurred in 36 %. Severe obstructive lesions (75 % or more) occurred predominantly in one or two arteries, while chronic obstruction of lesser degree (50–75 %) involved as a rule two, three or all four major arteries. Proximal and middle segments of the left anterior descending and right coronary arteries were the most common sites of chronic lesions. When chronic obstruction was simultaneously present in several coronary arteries, mostly it was a combination of left anterior descending and right coronary arteries.

In view of the data suggesting that natural progression of stenotic coronary atherosclerosis occurs predominantly in proximal and middle segments of left anterior descending and right coronary arteries, the presence of injury in only one of them, must alert one prospectively to the possibility of acute episodes of coronary heart disease.

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**CORONARY ARTERIOGRAPHIC
AND ELECTROCARDIOGRAPHIC
CORRELATES OF SUDDEN CARDIAC DEATH**

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While the mechanisms of sudden arrhythmic death are not clearly understood several studies from The Johns Hopkins University have recently implicated ischemia or infarction of the anteroseptal myocardial segment as important contributing factors in the genesis of ventricular fibrillation and tachycardia. These findings will be summarized in this report.

Sudden cardiac death or serious ventricular arrhythmias have been noted in several clinical situations including stable angina pectoris, prolonged rest pain as a complication of acute myocardial infarction during exercise electrocardiography and the induction of anesthesia for bypass graft surgery. The common arteriographic finding in most of these patients has been the presence of a 50 % or 70 % narrowing proximal to the origin of the first septal branch of the left anterior descending artery (LAD) either in the main left coronary artery (MLCA) or in the LAD segment proximal to this branch. Patients with a proximal narrowing in either of these two coronary segments can often be identified by simultaneous electrocardiographic changes noted in leads I and/or AVL and V2-V5 at the time of angina pectoris or acute myocardial infarction.

METHODS AND RESULTS

*Stable angina pectoris*¹¹

During the eight year period between 1960 to 1967 350 patients underwent selective coronary arteriography at the Johns Hopkins Hospital. Of this group 90 patients had at least one major coronary artery narrowed by 70 % or more, a technically satisfactory arteriogram to permit complete interpretation and did not have primary valvular or myocardial disease. The coronary arteriograms of these 90 patients were reviewed in a "blinded" fashion without knowledge of the patient's subsequent clinical course and each patient was judged to "suitable" or "not suitable" for coronary artery bypass surgery by 1977 criteria. Twelve patients were considered "not suitable" for bypass surgery (a). Nine were considered "non suitable" because of appreciable atherosclerotic plaque in the coronary segment distal to a significant narrowing at or beyond the usual bypass graft to coronary artery anastomotic site (b) three

patients were considered to have severe ventricular dysfunction because the cardiothoracic ratio on the P A chest film was greater than 50 % and left ventricular end diastolic pressure was more than 20 mm Hg, congestive left ventricular failure on clinical examination. Seventy-eight patients would be considered "suitable" for bypass surgery by current criteria. No patient went any operative procedure. Long-term follow up for periods up to 12 years (9.8 yrs) is available for these 78 patients. Fifty six of these 78 patients underwent double Master's exercise electrocardiogram test at the time of coronary arteriography.

Natural History. Eight years after coronary arteriography 26 of the 68 patients with 70 % or greater narrowing in the LAD-MLCA were dead compared to none of the patients who did not have at least a 70 % narrowing in either of these two vessels (P = 0.05). Among the 68 patients, three had a 70 % or greater narrowing in the MLCA, 65 had this narrowing in the LAD. Patients with a 70 % or greater narrowing in either LAD or MLCA were further analyzed on the basis of whether this narrowing occurred proximal or distal to the first LAD septal perforating branch. The "distal" narrowing was in the segment immediately beyond the first septal branch (Fig. 1). Eight years after arteriography 21 (52 %) of the 40 patients with a significant narrowing proximal to the first septal branch were dead while 6 (22 %) of the 28 patients with a narrowing distal to the first septal branch were dead (P < 0.05).

RISK OF DEATH AND LOCATION OF NARROWING IN LAD-MLCA

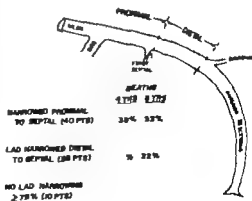


Figure 1

ise Electrocardiography The in on subsequent mortality of having a shive or negative exercise electrocardio- could be analyzed for the 56 patients had this test at the time of coronary

(Fig. 2) Mortality eight years arteriography was higher in those pa- is with a positive test compared with a rive test. ($P < 0.08$). The highest eight mortality was found in patients with a 4 or greater narrowing proximal to the t septal branch and a positive stress test. was no essential difference in eight mortality between those patients with a or greater narrowing distal to the first branch irregardless of whether the test was positive or negative and patients with a 70 % or greater narrow proximal to the first septal branch but a ve stress test.

proximal LAD narrowing and a posi- exercise ECG test appears to identify the up at highest risk of subsequent death. If assumption is correct that "viable myo- m is required for a patient to have a live stress ECG test, then the patient at risk of death would be the individual a proximal LAD narrowing and vi anteroseptal myocardium.

cardiac death Of the 78 patients suitable for bypass surgery by criteria, 31 have died of presumed dia causes up to 12 years after arteriogra- The circumstances of death are known patients. Using a definition of sudden death as death occurring within one r of being ambulatory stable and well 15 lens died a sudden cardiac death and 14 not. (Table 1) Of the 20 patients who died had a 70 % or greater narrowing proxi



Figure 2

mal to the first septal branch, 13 (65 %) died suddenly. Of the nine patients who died and had a 70 % or greater narrowing in the distal LAD only two (22 %) died suddenly. Thus 13 of 15 sudden cardiac death patients had a 70 % or greater narrowing proximal to the first septal branch of the LAD.

Arteriography in survivors of ventricular fibrillation or tachycardia¹

One hundred and seventeen patients who recovered from either ventricular fibrillation (65 pts) or ventricular tachycardia (52 pts) subsequently underwent coronary arteriography at The Johns Hopkins Hospital between 1970—1977. All were found to have coronary heart disease defined as one major coronary artery narrowed by at least 50 %. In these patients, the ventricular dysrhythmia had occurred as a complication of an acute myocardial infarction (92 pts) prolonged angina pectoris requiring admission to a coronary care unit but without evidence of myocardial injury (11 pts) or during a treadmill exercise electrocardiogram (14 pts). The coronary arteriogra-

Table 1
Relation of LAD narrowing and sudden cardiac death
(Follow-up to 12 yrs after coronary arteriography)

	Narrowing \geq 70% Proximal to First LAD Septal Branch	Narrowing \geq 70% in LAD Distal to First Septal Branch	No LAD Narrowing
Total Deaths	40	28	10
Sudden Deaths	20	9	2
Non-Sudden Deaths	20	19	8
Sudden Deaths	13 (65%)	6 (66%)	2 (100%)
	6 (30%)	3 (33%)	0
	1 (5%)	1 (11%)	0

phic findings in these patients were reviewed as to whether (a) there was a 50 % or greater narrowing in one two or all three major coronary arteries (left anterior descending, left circumflex or right) In 13 patients a 50 % or greater narrowing in the MLCA was counted as at least two vessel disease (b) there was a 50 % or greater narrowing in the LAD segment proximal to the first septal branch or in the main left coronary artery

The extent of disease (single double or triple vessel disease) found in these 117 patients is shown in Table 2. Also included is whether there was a 50 % or greater narrowing in either the LAD or the MLCA, and whether the LAD narrowing was proximal to the first septal branch. Twenty two (85 %) of the 26 patients with single vessel disease had significant disease limited to the LAD. In 18 (65 %) of these patients, the single significant narrowing was proximal to the first septal branch. In four it was distal to the first septal branch but proximal to a second large septal branch. Of the 117 patients 111 (95 %) had a 50 % or greater narrowing in either the LAD or MLCA, while 99 (85 %) had this narrowing proximal to the first LAD septal branch

Table 2
Location of $\geq 50\%$ Narrowing in VF/VT

Extent of Disease	PTS	LAD or Main left	Proximal LAD or Main left
Single	26	22	18
Double	26	24	19
Triple	65	65	62
	117	111 (95%)	99 (85%)

There is no appreciable difference in the coronary arteriographic findings between patients that had ventricular fibrillation prior to

Table 3
Arteriographic Findings in VF/VT

Extent of Disease	VF	VT
Single	14 (22%)	12 (23%)
Double	15 (23%)	11 (21%)
Triple	36 (55%)	29 (56%)
	65	52

Table 4
Arteriographic Findings in VF/VT

	VF	VT
Patients (No.)	65	52
SVD-LAD	10 (15%)	14 (26%)
LAD or MLCA Narrowed		
> 50%	60 (92%)	51 (98%)
Proximal LAD or MLCA Narrowed		
> 50%	53 (81%)	45 (86%)

arteriography and those that had tachycardia. (Tables 3 and 4) The extent coronary disease (single, double and vessel) as well as the extent of narrowing with single vessel LAD disease, a 50 % greater narrowing in the LAD or MLCA, those patients having this narrowing before the first septal branch is not significantly different between these two patient groups.

Anesthesia for bypass graft surgery¹²

Between 1969 and May 1973 269 patients underwent coronary artery bypass graft surgery at the Johns Hopkins Hospital. Ninety three (3 %) of these patients developed either atrial fibrillation or tachycardia during induction of anesthesia or prior to going cardiopulmonary bypass. Four of these patients had a 50 % or greater narrowing in the MLCA, four had a significant narrowing in the LAD proximal to the first septal branch and one had a significant narrowing just distal to the first septal branch but proximal to the second septal branch.

Survival after resuscitation of out-of-hospital ventricular fibrillation

Cobb and his associates⁴ have presented data on the two year mortality of 239 patients who were initially resuscitated from out-of-hospital ventricular fibrillation (Table 5). All patients were hospitalized and subsequent mortality after hospital discharge was examined by two criteria (a) whether the patient did or did not sustain a transmural infarction identified by a new Q wave or (b) whether the patient did or did not have myocardial "necrosis" defined as either a new Q wave or

	Patients	Deaths (2 years)
Q Wave	39	5 (13%)
Q Wave	200	76 (38%)
al Necrosis	78	16 (20%)
Myocardial Necrosis	97	46 (47%)
* Q wave and Alfa 1 LDH > Alfa 2 LDH		

of α 1 LDH that was greater than α 2 LDH. Seventy-one percent of patients who died during the two-year follow-up period died suddenly. When submortality during the two years after initial cardiac arrest was examined by two criteria, significantly higher mortality was found in the group of patients who did not develop a new Q wave or did not have evidence of myocardial necrosis compared to patients who did develop a new Q wave or evidence of myocardial necrosis. ($P < .05$). Among the patients who died, and the instances of death are known, the major cause (53/75 71%) were unexpected and occurred outside of hospital.

The authors strongly suggest that a patient rescued from ventricular fibrillation with evidence of an acute transmural infarction or myocardial necrosis is prone to current risk of sudden death, most likely ventricular fibrillation. Conversely myocardial infarction at the time of ventricular fibrillation may convey some protection against subsequent ventricular fibrillation.

In these patients appears to be associated with increased electrical instability.

Electrocardiographic recognition of significant left anterior descending narrowing¹

The twelve-lead electrocardiograms at the time of acute myocardial infarction (73 pts) or angina pectoris (33 pts) were examined in 106 patients with single vessel coronary artery disease. Single vessel disease was defined as one major coronary artery (left anterior descending, circumflex or right) narrowed by 70% or more while other arteries were narrowed by less than 40%. In the patients with acute infarction, only the appearance of new Q waves or ST segment shift of one millimeter or more (elevation or depression) or both, were noted. In those patients where the ECG was recorded during an episode of angina pectoris (either spontaneous or exercise induced), only reversible ST segment shift or T wave inversion or both were noted.

The ECG leads that showed new changes during infarction or reversible changes during angina are recorded in Table 6. If significant coronary disease is limited to one artery (e.g., the LAD), the changes will be found in the same leads whether the ECG is recorded during infarction or angina. No single ECG lead identifies disease in a specific vessel; the myocardial segment perfused by the left anterior descending is reflected as a combination of leads I, AVL and V1-V5; the myocardium perfused by the right coronary artery is reflected in leads II, III and AVF and the circumflex in leads II, III, AVF and V5-V6. All patients with circumflex disease had ECG changes in at least two of the three inferior leads (II, III, AVF) and three of ten also had changes in V5 or V6. Hence, if one examines the pattern or combination of ECG leads that are abnormal, it is usually possible to predict whether there is a significant

* 6

Changes in Single Vessel Disease

	PTS	I	AVL	V ₁	No. Patients with ECG Change in each lead								II	III	AVF
					V ₂	V ₃	V ₄	V ₅	V ₆						
D	60	26	41	44	49	44	4	34	24	1	2	1			
A	36	0	1	0	2	1	1	4	5	25	33	28			
C	11	0	0	0	2	1	1	4	5	10	11	10			

* Q + or ST shift. Angina: ST shift + or T wave inversion.

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two criteria, significantly higher mortality was found in the group of patients who not develop a new Q wave or did not have evidence of myocardial necrosis compared to patients who did develop a new Q wave or evidence of myocardial necrosis. ($P < .05$). Among the patients who died, and the circumstances of death are known, the major (53/75 71%) were unexpected and occurred in hospital.

The authors strongly suggest that a patient resuscitated from ventricular fibrillation without evidence of an acute transmural infarction or myocardial necrosis is prone to risk of sudden death, most likely from ventricular fibrillation. Conversely myocardial infarction at the time of ventricular fibrillation may convey some protection against subsequent ventricular fibrillation. Replacement in these patients appears to be associated with increased electrical stability.

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Table 6

ECG Changes in Single Vessel Disease

PTS	No. Patients with ECG Change in each lead										
	I	AVL	V ₁	V ₂	V ₃	V ₄	V ₅	V ₆	II	III	AVF
D 60	26	41	44	49	44	42	34	24	1	2	1
36	0	1	0	2	1	1	4	5	25	33	28
11	0	0	0	2	1	1	4	5	10	11	10

Q+ or ST shift Angina ST shift + or T wave inversion.

cant narrowing in the left anterior descend ing artery

Often it is also possible to predict by elec trocardiographic changes whether the signi ficant LAD narrowing is proximal to both the first diagonal and first septal branches. Based on other data, Griffith³ has proposed that the myocardium perfused by the first diagonal branch of the LAD is reflected in leads I and/or AVL and probably also V2—V3 while the myocardial segment perfused by the dis tal main stem is reflected in leads V2—V6 (Fig. 3) The patient who has simultaneous ECG changes in leads I and/or AVL and V2—V6 has myocardial ischemia or infarct in the myocardial segments perfused by the main stem LAD and the first LAD diagonal branch Simultaneous ischemia or infarction in both of these segments is usually due to a narrowing of the LAD proximal to or at the origin of the first LAD diagonal branch In more than 90 % of patients the first LAD diagonal branch comes off the LAD proxi mal to or at the same level as the first septal branch For this reason

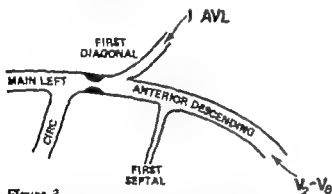


Figure 3

a narrowing proximal to the origin of the LAD diagonal branch is also proximal to the first septal branch Hence ECG changes in I and/or AVL and V2—V5 usually identify the patient with a significant narrowing in the LAD proximal to the origin of both the first diagonal branch and the first septal branch It should be noted that the magnitude of ECG changes in leads I and/or AVL is often less impressive than those found in the precordial leads (V2—V6)

All 73 patients with an acute anterior myo cardial infarction complicated by ventricular fibrillation or tachycardia had changes in leads I and/or AVL as well as the precordial leads An example of these ECG changes is shown in Figure 4 This ECG was recorded at

the time of CCU admission because of acute anterior infarction No significant tricular arrhythmias occurred at the initial hospitalization However on day this 62 year old male had a second prolonged episode of chest pain and de veloped ventricular tachycardia at that time Co

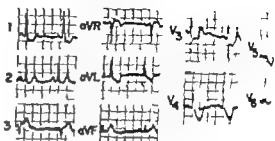


Figure 4

nary arteriography on the 12th hospital showed a 90 % occlusion in the LAD proximal to the origin of both the first and first septal branches.

DISCUSSION

The presence of a significant coronary ar terial narrowing in the main left coronary artery in the left anterior descending segment prox imal to the first septal branch appears to be a patient at "high risk" of subsequent death or serious ventricular dysrhythmia The data suggest this relationship have been observed in several clinical situations including: patients with stable angina pectoris and prolonged rest angina as well as a complica tion of acute infarction exercise electrocard iography and induction of anesthesia for by pass graft surgery Taylor and his assoc iates at Johns Hopkins have shown a similar cor relation between increased mortality follow ing survival from acute infarction and a 50% or greater narrowing proximal to the first LAD septal branch compared to other pa tients who survived infarction but did not have a significant narrowing before the first septal branch All of these postinfarction pa tients studied by Taylor et al lived at least 10 days after an acute infarction underwent re search coronary arteriography prior to hos pital discharge and were followed prospec tively on only medical therapy

There are at least two possible reasons why a proximal LAD narrowing might place a patient at high risk of ventricular fibrillation or tachycardia. One explanation is

myocardial segment in jeopardy of ischemia or infarction as a result of this proximal narrowing. A narrowing in this location usually impairs perfusion to three myocardial segments, namely the myocardium fused by the LAD diagonal, the main stem of LAD and the interventricular septum. Second explanation relates to ischemia or infarction of the interventricular septum per se and the His-Purkinje system that is included in this segment.

The role that "viable" myocardium has in the genesis of ventricular fibrillation or tachycardia is yet to be determined. In the natural history study cited previously the patients at highest risk of death (including sudden cardiac death) appear to be those with a positive stress ECG and a proximal LAD narrowing. In a group of patients with single vessel coronary disease, Griffith⁷ has suggested that a positive stress test identifies "viable" myocardium in the distribution of the obstructed coronary artery while a negative stress test probably correlates with "scarred" myocardium in the distribution of the obstructed artery. In the patients with single vessel disease, "viability" or "scarring" of the segment perfused by the obstructed coronary artery had been identified by either retained absent (akinesis) wall motion on left ventriculography. Certainly Cobb's data on survival after resuscitation of out-of-hospital ventricular fibrillation would suggest that the likelihood of a second episode of ventricular fibrillation or death was significantly higher in patients who did not have a transmural infarction or myocardial necrosis at the time of the initial cardiac arrest. Continued myocardial "viability" as contrasted with myocardial "scarring" identified the patient at high risk of subsequent death.

If further prospective studies show a significant narrowing proximal to the left anterior descending first septal branch correlates with high risk of death, especially sudden cardiac death, then appropriate non-invasive tests to identify these patients must be developed. The use of Thallium-201 myocardial perfusion imaging or recognition of simultaneous ECG changes in I and/or AVL and II-V6 may prove useful. Finally therapeutic regimens including specific antiarrhythmic therapy or coronary artery bypass surgery must be evaluated in patients whose

coronary artery and left ventricular anatomy would place them at "high risk" of sudden cardiac death.

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THE EXERCISE ECG AND RELATED PHYSIOLOGICAL DATA AS MARKERS OF CRITICAL CORONARY ARTERY LESIONS

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The single most important determinant of the prognosis in acute myocardial infarction is the relative magnitude of muscle loss, i.e. the ratio between the mass of the irreversibly damaged and the total left ventricular myocardial muscle mass (1). The prognosis in chronic ischemic heart disease may also be determined by the percentage of left ventricular mass that has been lost or is at high risk of sustaining ischemic injury. Recent studies by Kalbfleisch and Hort (2) indicate that the left main coronary artery normally supplies nearly two-thirds (64 %) of the left ventricular muscle mass and the left anterior descending artery about 40 % (Table 1). These anatomical data are in agreement with the current clinical view that patients with left main or proximal anterior descending lesions join patients

Table 1
Relative Size of the left Ventricular Arterial Supply Areas

Per cent of total left ventricular myocardium supplied by the left anterior descending (LAD) circumflex (LCX) and right coronary (RCA) arteries

Arterial pattern	N	Per cent supplied by		
		LAD	LCX	RCA
Left	19	44	40	16
Balanced	129	42	21	37
Right	23	39	15	46
Total	171	42	22	36

From Kalbfleisch and Hort (1977)

with three vessel disease in a group of unfavorable prognosis (3).

We have explored the extent to which the sub-groups with poor prognosis may be identified by data derived from routine clinical exercise test. We asked the following specific questions

1. To what extent does the exercise ECG provide specific information on the localization of arterial lesions?
2. Can the combined electrocardiographic and physiological exercise test data identify patients with three vessel, left main or proximal left anterior descending artery disease?

MATERIAL AND METHODS

Several subsets of patients were examined. All had in common a history of chest pain. Patients with congenital valvular or myocardial disease were excluded as were patients with recent (less than two months) myocardial infarction. Left ventricular coronary cineangiograms were obtained using standard techniques (Sones or Judkins).

The exercise test was uniformly a continuous upright bicycle exercise with three minute work periods of progressively heavier loads. The test was carried to a symptom limited maximal level (4). Reasons for discontinuing exercise included silent but unequivocally abnormal ST segment displacement. Peak work load was measured in kilopondmeters per minute (kpm/min). Oxygen uptake was estimated by the method of Suddell as modified by Sanne (5, 6) related to age and sex specific standards (7) and expressed as per cent of expected normal values. Indirect arterial pressures were recorded by a semi automatic device (Colson sphygmomanometer Narco Systems, Inc.) and respiratory rate by a nasal thermistor.

The ECG analysis was based on a modified Frank lead system. Amplitude measurements were referred to the level of the PR segment immediately before the onset of QRS. ST amplitudes (Fig. 1) were measured at nine points defining eight subsegments of equal duration.

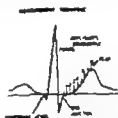
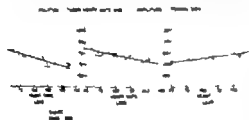


Fig. 1 ECG Measurement Technique
ST is characterized by amplitude measurement. Frank leads X, Y and Z at nine points dividing the interval End of QRS—Peak spatial magnitude of T into eight subsegments of equal duration. The zero reference level is the level of the P-R segment immediately before the onset of QRS.

duration between the end of QRS and peak T. ST₂ distributions, i.e. amplitudes at one-fourth the distance between end of QRS and peak T were studied in detail. The ECG methods including computer programs for wave recognition, averaging, and measure-

ent have been fully described elsewhere (8)

Normal reference data were obtained from group of 110 subjects, 65 men and 45 women, 18 to 29 years old. The normal data included bi-variate frontal and horizontal sine distributions in a subgroup of 40 men and women (9). Analysis of the normal ECG (Fig. 2) indicated that heart rate is a major determinant of the normal ST amplitude (10). Linear correlation coefficients for lead X (left to right) and Y (inferior superior) range from 0.69 at the QRS-ST junction to 0.4 at ST 4 i.e. at the half way mark between end of QRS and peak T. The heart rate relations were weaker in lead Z (posterior).



2. Relation between Frank lead X, Y and Z ST amplitudes and heart rate during exercise in normal men and women.

er anterior). The strength of the orthogonal leads is lower than that of the standard chest leads. An ST amplitude of 3/4 mm in lead X is the equivalent of 1 mm in V_4 (11). The normal range in leads X and Y at maximal heart rates is 0.5 to 0.7 mm in men. Anterior displacement (corresponding to ST elevation in leads V_4) up to 1.5 mm is a normal finding while any posterior displacement is abnormal.

There were significant sex differences with respect to the normal ST response to exercise (Fig. 3). Women had more prominent superior ST displacement than men i.e. larger ST depression in lead Y with a lower limit of 1.2

This difference may account for the high rate of false positive ECG responses in men when conventional uniform criteria are applied.

RESULTS

The validity of the normal reference data for ECG analysis was tested in a series of 20 patients (mean age 47) with normal coronary arteries, normal left ventricular catheteriza-

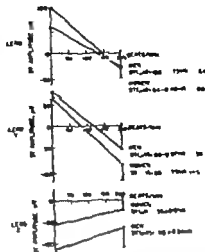


Fig 3 Relation between ST 2 amplitude and heart rate in normal men and women. Linear regression.

tion data, and on no digitalis. One of the twenty exceeded the lead heart rate sex specific limits as defined by the 5th and 95th percentile of the ST distributions in the young normal reference population.

The relation between the extent of coronary artery disease and the characteristics of the ST segment during exercise was investigated in a series of 67 patients with at least one 50% diameter reduction in a major vessel. A summary of the basic clinical data appears in Table II.

Table II
ECG Series — General Characteristics
Extent of Coronary Artery Disease

	1 Vessel	2 Vessels	3 Vessels	Left Main	Total
No. of patients	24	20	16	7	67
Per cent	36	30	24	10	100

Age: Mean age 50.5 ± 1.2 years, Range 32 to 67
Distribution of Single Vessel Disease
LAD 13, LCX 4, and RCA 7 patients.

1 Effect of the site of coronary artery lesions on the spatial characteristics of the ST segment during exercise

The basic relationship between the site of arterial lesions and the ST segment characteris-

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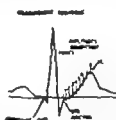


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positive electrode, the normal and the ischemic myocardium. Transmural anterior and/or subendocardial posterior ischemia will produce anterior ST displacement, i.e. elevation in standard leads V_{1-2} , while transmural posterior and/or subendocardial anterior ischemia will result in posterior displacement. Furthermore, it seems inevitable that localization of the ischemic zone will vary for any given site of an arterial lesion in severity of the obstruction, presence or absence of collateral circulation and normal relations with respect to the geometry of the ventricular supply areas (2). The present series is all but nevertheless demonstrates that the fixation of specific areas of ischemia in single-vessel disease cannot be accurately assessed from the spatial characteristics of ST segment.

The extent of coronary disease as a determinant of ST segment characteristics

The relation between the extent of coronary artery disease and the prevalence of ST amplitude abnormalities at rest and during exercise is illustrated in Table III. The high rate of ST abnormalities at rest among patients

Table III

Prevalence of ST Abnormalities at Rest and during Exercise (Per Cent) Related to the Extent of Coronary Artery Disease

	Diseased Vessels			
	1	2	3	Left main
at rest	12.0	10.5	62.5	28.5
during exercise	74.0	79.0	93.8	83.5
	25	19	16	7

with three vessel disease is in agreement with previous findings (9). The increasing likelihood of abnormal ST response to exercise with increasing extent of coronary artery disease is well documented (12, 13, 21-23) in the literature (Fig. 6).

Lead by lead analysis of the relation between heart rate and ST amplitude (Fig. 6) called no specific patterns. The average peak heart rate for patients with two and three vessel disease was 129 beats per minute.

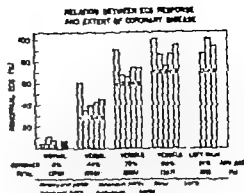


Fig 6 Relation between the rate of abnormal ECG responses to exercise and the extent of coronary artery disease

Mean values were lower in left main disease (104 ± 5 beats per minute) and higher in single vessel disease (137 ± 5 bpm). The intra-group variations were large and the inter-group differences were not statistically significant.

Evaluation of the spatial distribution of ST amplitudes during peak exercise (Fig. 7) demonstrated that 19 of 23 patients (83%) with left main or three vessel disease had an ST vector during exercise located in the right upper anterior octant compared to 24 of 44 (55%) of the patients with one or two vessel disease ($p < 0.01$ chi-square). The homogeneous distribution in three vessel and left main disease is consistent with widespread left ventricular subendocardial ischemia with an ST vector 180° away from the free wall. The more variable ST distributions in single and two vessel disease probably reflect the pre-

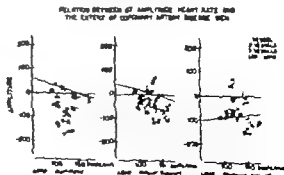


Fig 7 Relation between Frank lead ST 2 amplitude heart rate during exercise and the extent of coronary artery disease in 42 men. Dotted lines correspond to the 5th and 95th percentiles of the normal male ST distribution

tics was examined in a subgroup of 24 patients with single vessel disease. The ECG response was abnormal in 13/14 patients with LAD lesions compared to 1/4 with circumflex and 5/7 with RCA lesions. The numbers are small but tend to support the findings by McHenry et al (12) who concluded that RCA and circumflex lesions frequently are electrocardiographically silent. Others (13) have been unable to demonstrate any differences between subgroups of patients with single vessel disease.

There is little truly quantitative information on the spatial relationship between the anatomical site of the arterial lesion and the ST segment displacement. Most of the available spatial data deal with ST elevation, i.e. ST displacement to the left, inferiorly or anteriorly. Exercise-induced ST elevation is a common feature during the early recovery stage after myocardial infarction (14, 15). ST elevation is then usually found in leads with Q waves and is consistent with per infarction transmural ischemia. Old myocardial infarction associated with significant wall motion abnormalities is also found in a large number of patients with ST elevations during exercise in chronic coronary disease usually in the absence of angina pectoris (16). Data presented by Fortuin and Friesinger (17) suggest that exercise induced ST elevation also in the absence of previous myocardial infarction has a strong localizing power and usually is associated with a severe proximal stenosis of a major artery i.e. the anatomy of the classical Prinzmetal syndrome. Hegge et al (18) also compared electrocardiographic and angiographic data and found close agreement between the site of arterial lesions and the presence of ST elevation in anterior precordial leads. Simoons (19) related the direction of the ST segment to the anatomical site of wall motion abnormalities and found a better correlation in inferior lesions. We have observed several patients with anterior ST displacement during exercise and severe proximal LAD obstructions (Fig. 4) but a critical analysis of our series of 24 patients with single vessel disease (Fig. 5) demonstrated that relationship between the site of the arterial lesion and the direction of the ST vector is complex.

Significant leftward and/or inferior ST displacement (ST elevation in standard late

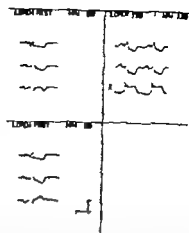


Fig. 4 Transient ST elevation (anterior-right superior ST displacement) during exercise by a patient with a single vessel coronary disease i.e. a 95 diameter reduction of the proximal left anterior descending artery. Positive polarity lead X left. Y anterior and Z anterior. Calibration 200 msec/0.5 Load given as kpm/min

ral or vertical leads) was seen in only patients and each had a remote transmural infarction and arterial lesions at the sites. The majority of the patients with LA lesions, or 9/13 had some degree of a displacement, but three patients had posterior displacement. Patients with RCA lesions were evenly divided between anterior posterior displacement.



Fig. 5 Spatial ST distribution in single vessel coronary disease. ST amplitudes measured at 2/8 the interval and QRS-peak T during symptom-limited maximal exercise. N=27

The overlap of ST sites in LAD and RCA lesions is not surprising. The injury current is generated in the boundary zone separating normal and ischemic myocardium (20). The direction of the ST segment displacement is determined by the spatial relation between

4 Y
 of the Extent of Coronary Artery Disease and Beta Blockade on the Response to Exercise
 as Values Standard Error

Parameter	B-Block	No Disease	1 Vessel	2 Vessels	3 Vessels	Left Main
HR	-	148.6	142.6	134.10	177.6	128.5
	+	118.7	131.11	104.10	108.13	110.7
SBP	-	175.19	170.10	169.11	159.10	153.13
	+	170.10	172.9	150.10	153.19	145.11
MAP	-	262.17	241.15	228.23	200.15	198.23
	+	206.22	227.25	158.24	182.25	157.24
Stroke Volume	-	62.4	54.3	60.3	50.4	52.6
	+	69.7	62.6	51.6	37.5	36.5
of beats	-	16	6	5	11	9
	+	19	10	7	15	5

Rate-pressure product (Heart Rate \times Syst. Blood Pressure $\times 10^{-3}$).

Peak oxygen uptake as per cent of normal age- and sex-specific mean values (7).

ten subgroups with respect to age. Forty per cent of the patients with coronary disease and 44 % of the patients with normal coronary arteries were taking propranolol at the time of the study.

As expected (27), hemodynamic and non-invasive physiological data (Table V) demonstrated a progressive functional impairment with increasing extent of coronary artery disease. Differences between patients with three vessel or left main disease and patients with normal arteries with single vessel disease were significant at least at the five per cent level (*t*-test). Patients with left main and three vessel disease had similar exercise test data and three vessel disease was associated with more prominent hemodynamic abnormalities, including wall motion abnormalities (*p* < 0.01).

All physiological measurements demonstrated wide intra-group variations and considerable inter-group overlap. No single data point provided any separation of clinical value.

Beta blocking agents tended to obliterate inter-group differences with respect to heart rate and rate pressure product (heart rate \times systolic blood pressure $\times 10^{-3}$) but differences with respect to peak work load and peak oxygen uptake persisted. Clinical consideration often necessitates evaluation of the patient during continued beta-adrenergic blockade.

We therefore selected work load as an index of cardiovascular functional capacity. Use of estimated oxygen uptake normalized for body weight proved ineffective in our population since no less than 85 % of the female and 60 % of the male patients were classified as grossly overweight by their ponderal indices.

The combined requirement of signs of ischemia during the exercise test, i.e. ST segment abnormality and/or typical angina pectoris, and a low work capacity i.e. peak work load 400 kpm/min in men and 250 kpm/min. in women had considerable dis-

Table VI
 Identification of Patients with 3 Vessel and Left Main Disease

Criteria for Positive Test: Angina and/or ST Abnormality at Low Work Load (≤ 400 kpm/min. in men and ≤ 250 in women)

		No. of Patients with		
		0- Vessel	3 Vessel	Left Main
Ischemia at Low Work Load	+	4	12	11
	-	65	14	3
Total		69	26	14

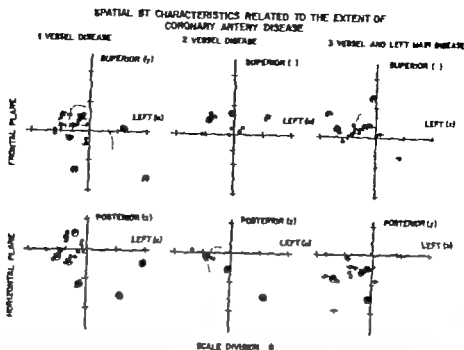


Fig 8 Spatial ST characteristics related to the extent of coronary disease. Circles indicate remote myocardial infarction and crossbars (right panel) patients with left main disease

sence of one or more discrete areas of subendocardial and/or transmural ischemia.

III Relation between the extent of coronary artery disease and combined electrocardiographic and physiological exercise test findings

It has been suggested by several authors that ST depression exceeding 2 mm (0.2 mV), identifies left main disease with a relatively high accuracy. Analysis of published data (22, 24-26) in terms of sensitivity, specificity and predictive value (Fig. 8) indicates a moderately effective diagnostic performance. However, the use of silent but definite ST abnormality of the ischemic type as an

exercise test endpoint—which is considered prudent in many laboratories, including our own—obviously invalidates the use of ST depression as an endpoint. Furthermore, the severity of the angina accepted as an end point is likely to vary with the variable lead strength of no monitor leads or lead systems, another uncontrolled element. Alternative criteria are therefore desirable.

We examined the discriminatory power of the complete exercise test data. The significance of the ECG response combined with measurements relating to physical work capacity, heart rate, blood pressure, and limiting symptoms and signs was tested in a study of 108 patients. Each patient had a history of chest pain and complete angiographic and exercise studies. Thirty-six patients were free from significant coronary artery disease. Basic clinical characteristics are listed in Table IV. There were no significant differences

Table IV
Clinical correlation Series-Basic Patient Characteristics
Extent of Coronary Artery Disease

	None	1 Vessel	2 Vessels	3 Vessels	Left Main	Total
Men	13	10	6	14	8	51
Women	23	9	7	12	6	57
Total	36	19	13	26	14	108

Mean Age 49 years
Documented Myocardial Infarction 1/36 patients without and 35/72 with coronary artery disease.
Current Propranolol Therapy 16/36 patients without and 31/72 with coronary disease

In summary we have demonstrated a limited ability of the exercise ECG to localize myocardial ischemia to specific arterial distribution areas. The relatively tenuous anatomical relationship between the site of any arterial lesion and the localization of discrete areas of myocardial ischemia, combined with a 180° change in the direction of the ST segment placement when subendocardial ischemia becomes transmural, most likely accounts for the widely scattered ST distributions in one and two vessel disease. Patients with left main or two vessel disease showed a remarkably uniform ST distribution consistent with global left ventricular subendocardial ischemia. The demonstration of ischemia at a low work load level appears to be a useful clinical sign of left main and three vessel disease. We are unable to identify proximal LAD lesions from the exercise test data.

The combined results emphasize the role of exercise testing as an independent method as a means of quantifying the physiological impact of coronary disease rather than as an alternative to angiography as a means of obtaining precise anatomical data. Recent studies (30, 31) strongly suggest that the exercise test results have considerable prognostic significance which may be at least partially unrelated to the anatomical extent of the arterial disease.

Table VII
Myocardial Ischemia at Low Work Load

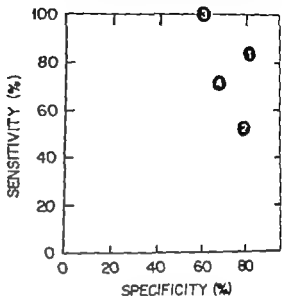
Diagnostic Power	Left Main and 3 V vs. 0-2 V	Left Main vs. All Others
Specificity	0.94	0.80
Sensitivity	0.58	0.79
Predictive Value Neg. Test	0.79	0.96
Predictive Value Pos. Test	0.85	0.41

crimutory power as demonstrated in Tables VI and VIII. All 36 patients without significant arteriographic disease had a negative result. The predictive values indicate that in the population under study 8/10 patients with a negative test were free from three vessel or left main disease while 8/10 patients with a positive test had three vessel or left main disease. The specificity of the test when used to differentiate patients with left main disease from all others was lower but the predictive value of a negative test was high 0.96. Thus failure of the exercise test to produce ischemia at a low work load level provides strong evidence against left main disease.

Corresponding results were obtained by Nixon et al (28) in a different population (n=75) studied at the Dallas VA Hospital. They used the identical criteria to separate male patients with left main disease from patients with two and three vessel disease. Sensitivity and specificity were 0.64 and 0.80 and the predictive value of a positive test 0.25 and of a negative test 0.96 at an overall prevalence of left main disease of 9%.

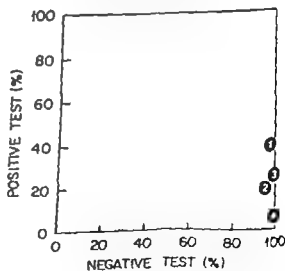
A total of 29 patients in our series had a proximal LAD diameter reduction of $\geq 90\%$. A majority or 19 patients had three vessel or left main disease. The presence of severe proximal LAD lesions was associated with a slightly lower functional capacity than in patients with no or lesser degrees of LAD disease and comparable RCA and circumflex lesions. However the functional impairment was not of sufficient degree to satisfy the criteria that identified patients with left main and three vessel disease.

**SENSITIVITY AND SPECIFICITY
ST DEPRESSION ≥ 2 MM.-LEFT
VS OTHER SIGNIFICANT
CORONARY ARTERY DISEASE**



1 Kohnen et al (1973) 2 Borstel et al (1974)
3 Charbon et al (1975) 4 Cohen et al (1975)

**PREDICTIVE VALUE OF
ST DEPRESSION ≥ 2 MM.-LEFT MAIN
VS OTHER SIGNIFICANT
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3 Demots et al (1973) 4 Cohen et al (1975)

**Fig 9 Diagnostic power of ST depression 2 mm
in left main coronary disease**

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8.

EXERCISE TESTING IN THE DIAGNOSIS OF CORONARY LESIONS PROXIMAL TO THE FIRST DIAGONAL BRANCH OF LAD

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From the Dept of Cardiology Sahlgrenska Hospital, Göteborg, Sweden.



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The objective of this report is to present some preliminary data from a study in progress aiming to investigate the above problems.

MATERIAL

The material consists of 15 patients who were angina pectoris not responding to medical therapy. Coronary bypass surgery was considered and they were admitted for evaluation. 14 of them were males and one female (No. 11) with the age ranging between 36 and 64 years (mean 54). The patients were kept under usual drug therapy at the examination. All of them were receiving β -blockers and seven had digitalis. Additional clinical data are given in Table I.

METHODS

Exercise test was performed on bicycle ergometer with step wise increasing work load. The duration of each step was one minute.

Table I
Summary of history and ECG at rest.

Group No	Case No	Age yrs	History					ECG at rest		
			MI	HT	Dig	Diu	Q-waves	ST-depression† I/aVL	III,aVF	CH ₂₄
			No							
I Q-wave in I/aVL V_{1-3}	1	54	1	—	—	—	I/aVL	+	—	+
	2	57	1	—	+	—	V_{2-3}	+	—	+
	3	54	2	—	+	+	V_{2-3}, aVF	—	+	+
	4	55	1	+	+	+	V_{2-3}	+	—	+
II ECG at rest normal. Pos. ET	5	45	1	—	—	—	—	—	—	—
	6	36	0	—	—	—	—	—	—	—
	7	60	0	—	—	—	—	—	—	—
	8	60	1	—	—	—	—	—	—	—
	9	51	0	—	+	—	—	—	—	—
III ECG at rest pathol Group I not included	10	64	0	+	+	+	—	—	+	—
	11	57	0	+	—	+	—	+	+	+
	12	54	2	—	+	—	III,aVF	+	+	+
	13	56	1	—	+	+	III,aVF	—	+	+
	14	56	0	—	+	—	LBBB	—	—	—
IV ECG at rest and ET normal	15	56	0	+	—	—	—	—	—	—

Abbreviations: MI = Myocardial Infarction HT = Hypertension Dig = Digitalis therapy Diu = Diuretic therapy CH₂₄ = Bipolar Chest Head leads ET = Exercise test.
† Presence of (or at work increase of) ST-depression $> 1/2$ mm. At rest biphasic T wave with first phase negative $> 1/2$ mm is included.
(+) Presence of (at work development of) upward-sloping ST-depression $> 1/2$ mm 60 msec after j-point.
— No ST Depression or ST-depression $< 1/2$ mm.

The exercise was discontinued at the point intolerable chest pain or fatigue. Heart rate was calculated from ECG every minute and tonic blood pressure was measured with cuff method at the end of each step. ECG was recorded continuously during exercise, immediately after stopping the exercise and 5 minutes later.

To minimize the disturbance of artefacts the extremity ECG leads the electrodes of which were placed on spina scapulae and crista iliaca respectively. The indifferent electrode of the bipolar precordial leads was fixed on the forehead (CH). The ECG signals sampled by the computer during the last seconds of each exercise step in order to obtain noise reduction and averaging of the signal (5).

Coronary arteriography and left ventricular angiography were performed 1–2 days after exercise testing. The arteriograms were coded according to the American Heart Association grading system (6). The proximal LAD lesions was thus defined as a significant obstruction (at least 50%) localized proximally to and including the origin of first major perforator septal branch. As a rule first diagonal artery was within this area. If not, this was specially noted.

RESULTS

The main results are listed in Table I and II. Three groups of patients could be distinguished. Group I included patients with ECG signs of previous transmural anterior wall infarction. Group II patients had normal ECG

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Study of ECG during exercise and coronary angiogram.

Case No.	ECG during exercise			Coronary arteriography §§				
	Development or increase of ST-depression. §			Main LCA	Prox LAD	Dist LAD	LCx	RC
	I/aVL	III,aVF	CH ₂₄					
1	+	+	4-5	-	+	+	-	+
2	-	+	1-2	-	++	-	-	-
3	-	+	0	-	++	-	+	+
4	-	+	1-2	-	-	++ (M)	-	-
5	+	(+)	2-3	-	++	-	+	-
6	+	(+)	2-3	-	++	-	-	++
7	+	-	2-3	-	++	-	-	+
8	+	-	2-3	-	+	+	-	++
9	+	(+)	2-3	-	++	-	++	-
10	+	+	3-4	-	+	+	+	++
11	-	+	2-3	-	-	+	+	+
12	-	+	1-2	-	-	-	+	+
13	+	-	1-2	-	-	-	-	+
14				+	-	-	-	+
15	-	-	0	-	-	-	-	-

Abbreviations and definitions: *Main LCA* = Main Left Coronary Artery
1. *LAD* = Proximal segment of Left Anterior Descending Artery

1. LAD = Proximal segment of Left Anterior Descending Artery including origin of first major forer septal branch and, if not commented first diagonal artery

LAD - Segment of LAD distal to origl of first major perforator septal branch.
= Left Circumflex artery.

- Left Circumflex artery

- Right coronary artery

As in Table I. For CH_2 during exercise, ST-depression in mV
++ 99-100% means of measurement.

+ 99-100% stems of main stem

- + $\geq 50\%$ of main stem or major branch
- $< 50\%$ main stem or major branch

< 50% stenosis or normal artery

first diagonal artery originates from main left coronary artery

Evidence presented here and elsewhere by Griffith and co workers and the original data of Janushkevichius et al presented here by Bloozhas implicate the proximal part of left anterior descending artery (LAD) as the most frequent site of coronary stenosis associated with sudden cardiac death. Depolarization changes in electrocardiographic leads I/aVL and V₁₋₄ during episodes of acute ischemia strongly suggest the presence of such lesions (1,2). It is, however, unknown how sensitive and specific these ECG alterations are. Do these ECG changes carry equal significance if provoked by exercise test?

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			MI	HT	Dig	Diu	Q-waves	ST-depression § I/aVL	III aVF	CH ₂₄
			No							
I Q-wave in I/aVL V ₂₋₃	1	54	1	—	—	—	I/aVL	+	—	+
	2	57	1	—	+	—	V _{2,3}	+	—	+
	3	54	2	—	+	+	V _{2,3} aVF	—	+	+
	4	55	1	+	+	+	V _{2,3}	+	—	+
II ECG at rest normal, Pos. ET	5	45	1	—	—	—	—	—	—	—
	6	36	0	—	—	—	—	—	—	—
	7	60	0	—	—	—	—	—	—	—
	8	60	1	—	—	—	—	—	—	—
	9	51	0	—	+	—	—	—	—	—
III ECG at rest pathol Group I not included	10	64	0	+	+	+	—	—	+	—
	11	57	0	+	—	+	—	+	+	+
	12	54	2	—	+	—	III aVF	+	+	+
	13	56	1	—	+	+	III aVF	—	+	+
	14	56	0	—	+	—	LBBB	—	—	—
IV ECG at rest and ET normal.	15	56	0	+	—	—	—	—	—	—

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(+) Presence of (at work, development of) upward sloping ST-depression > 1/2 mm 60 msec after j-point.

— No ST Depression or ST-depression < 1/2 mm

if the load increase was 10 watts per step (3). The exercise was discontinued at the point tolerable chest pain or fatigue. Heart rate was calculated from ECG every minute and aortic blood pressure was measured with cuff method at the end of each step. ECG was recorded continuously during exercise, and only after stopping the exercise and a minutes later.

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1	+	+	4-5	-	+	+	-	+
2	-	+	1-2	-	++	-	-	-
3	-	+	0	-	++	-	+	+
4	-	+	1-2	-	-	++ ⁹⁹	-	-
5	+	(+)	2-3	-	++	-	+	-
6	+	(+)	2-3	-	++	-	-	++
7	+	-	2-3	-	++	-	-	-
8	+	-	2-3	-	+	+	-	++
9	+	(+)	2-3	-	++	-	++	-
10	+	+	3-4	-	+	+	+	++
11	-	+	2-3	-	-	+	+	+
12	-	+	1-2	-	-	-	+	+
13	+	-	1-2	-	-	-	-	+
14	-	-	-	+	-	-	-	+
15	-	-	0	-	-	-	-	-

Abbreviations: Main LCA = Main Left Coronary Artery

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Dist LAD = Segment of LAD distal to origin of first major perforator septal branch.

1 Left Circumflex artery

2 Right coronary artery

In Table I For CH₂₄ during exercise, ST-depression in mm.

++ 99-100% stenosis of main stem

+ 50% stenosis of main stem or major branch

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Exercise test was performed on bicycle ergometer with step wise increasing workload. The duration of each step was one minute.

Table I
Summary of history and ECG at rest.

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			No							
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	2	57	1	—	+	—	V_{2-3}	+	—	+
	3	54	2	—	+	+	V_{2-3} aVF	—	+	+
	4	55	1	+	+	+	V_{2-3}	+	—	+
II ECG at rest normal Pos. ET	5	45	1	—	—	—	—	—	—	—
	6	36	0	—	—	—	—	—	—	—
	7	60	0	—	—	—	—	—	—	—
	8	60	1	—	—	—	—	—	—	—
	9	51	0	—	+	—	—	—	—	—
III ECG at rest pathol. Group I not included	10	64	0	+	+	+	—	—	+	—
	11	57	0	+	—	+	—	+	+	+
	12	54	2	—	+	—	III aVF	+	+	+
	13	56	1	—	+	+	III aVF	—	+	+
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 (+) Presence of (at work, development of) upward-sloping ST-depression $> 1/2$ mm 60 msec after J-point.
 — No ST Depression or ST-depression $< 1/2$ mm.

Lack of ischaemic ST-depression during exercise in leads I/aVL and CH₂₋₄ correctly indicated that there was no proximal LAD lesion in four out of six patients. In two patients the exercise test was falsely negative. One patient had abnormal resting electrocardiograms and was on digitalis or a diuretic and digitalis respectively. It is well known that cardio-active drugs and abnormal resting ECG negatively influence the validity of exercise ECG to predict angiographically demonstrable coronary lesions.

at rest but developed ST depression in leads I/aVL and CH₂₋₄ on exercise. Group III consisted of patients with various ECG abnormalities excluding those with the anterior wall Q waves classified separately into group I. Finally there was only one patient with normal ECG at rest and on exercise.

All patients except one (No. 13) developed severe chest pain during exercise test. The one who was free of pain during exercise developed it after exercise a usual type of response in this patient.

Maximal heart rate varied between 94 and 146 (mean 108) only one patient reaching 146 while the maximal heart rate in the remaining patients did not exceed 115. There were no marked differences in maximal heart rate between the groups or between patients with different degree of vessel involvement.

There was no difference in maximal systolic blood pressure response or maximal work load. The maximal systolic blood pressure ranged between 150 and 210 mm Hg and maximal work load between 60 and 160 watts.

The patient with a main stem lesion (No. 14) did not increase heart rate during the last five steps of exercise a sign not seen in other cases. His systolic blood pressure increased however in the usual fashion.

In group I there was one patient (No. 1) with a pathological Q-wave in leads I/aVL but not in the precordial leads. There were also three patients with Q-waves in the precordial leads but not in I/aVL. The ST-depression during exercise varied in this group. It was most marked in patient No. 1 with Q in I/aVL.

The patients of Group II who had normal ECG at rest responded to exercise with 1/2 mm or more ischemic ST-depression in lead I and much more marked ST-depression in the precordial leads. These ST-changes were present immediately (15–20 sec.) after exercise in all patients. No changes could be recorded in the majority of the patients four minutes later.

The patients belonging to group III showed one or other types of ECG abnormalities at rest and all of them except for case No. 14 had digitalis or/and diuretic treatment in addition to beta blockers. There was only one patient with proximal LAD stenosis (No. 10) in this group and he showed progress of

ST-depression in lead I/aVL and CH₂₋₄ during exercise. Similar ECG response to exercise although less pronounced, was also in case No. 13 who had a significant stenosis of right coronary artery and 50 per cent obstruction in the proximal I.

In case No. 11 with a pronounced ST depression in precordial leads but no leads I/aVL the arteriogram disclosed a stenosis beginning in proximal LAD with than 50 per cent obstruction and progressing to more than 50 per cent obstruction in the distal part of LAD possibly including the first diagonal artery. Patient No. 12 with two old posterior infarctions showed ST-depression in leads I/aVL and had significant disease of proximal LAD. Patient No. 14 had left bundle branch block, making further interpretation of ST-changes impossible. abnormal heart rate response to exercise was of diagnostic value.

Patient No. 15 had a normal ECG at rest and on exercise. The coronary arteriogram was also normal.

DISCUSSION AND CONCLUSIONS

First of all it should be emphasized that the computer program for noise reduction, stabilization of base line and ECG averaging was highly effective permitting a convenient interpretation and reading of all leads at rest and during exercise. ST segment deviation from the reference level by 0.5 mm or more was easily recognized and measured.

Occurrence or progression of already existing ST-depression during exercise in leads I/aVL and CH₂₋₄ were predictive of significant (> 50 per cent) proximal LAD stenosis in seven out of eight patients. Only one false positive case appeared to be present, but actually this patient (No. 13) also had a proximal LAD stenosis which was judged to be insignificant (< 50 per cent).

**NON INVASIVE DETECTION AND EVALUATION
OF THE FUNCTIONAL SEVERITY OF
CORONARY ARTERY DISEASE
THE ROLE OF RADIONUCLIDE CINEANGIOGRAPHY
DURING EXERCISE**

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Kenneth M. Kent, M.D., Ph.D.,
Bonnie Mack, M.S., and
Stephen E. Epstein, M.D.
With the technical assistance of
Susan Parkes, B.S.**

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EFFECT OF EXERCISE ON LEFT VENTRICULAR FUNCTION CORONARY ARTERY DISEASE

REST



ED

ES

DIFF

EXERCISE



ED

ES

DIFF

Figure 2 Discontoured selected frames, in sequence, of a radioisotope cineangiogram taken in a patient with three-vessel coronary-artery disease at (upper) and during maximal exercise (lower).

(Abbreviations as in Figure 1). (Figure and legend reprinted by permission from the New England Journal of Medicine 296: 841, 1977).

ies of images (framing rate up to 100 mes/sec). These images, which reside in core memory of the computer, span the trace cardiac cycle. While data are being stored, the images can be displayed as an end-loop, flicker free movie (Figure 1, 2). It allows immediate access to qualitative information regarding spatial and temporal relations of the cardiac chambers. Visually interpretable information is produced within seconds of the onset of data collection and statistically reliable results within one to minutes.¹ Thus, data collection is sufficiently rapid to permit the procedure to be applied to studies during exercise induced ischemia and angina which might preclude prolonged exercise and imaging.

While the cardiac images are being created, the computer simultaneously analyzes the stored data to produce a time-activity curve with high (10 msec) temporal resolution (Figure 3). Corrections for background activity are made as previously described.¹ Since radioactivity is proportional to blood volume, after correction for background the time-activity curve in fact represents a measure of left ventricular volume versus time. Therefore, once the physician has identified the left ventricle in the end-diastolic movie frame, quantitation of left ventricular volume over time can be performed. In order to obtain statistically reliable information,

the computer sums the radioactivity in the ventricle during many beats. After each cardiac cycle the length of the RR interval is automatically examined to determine if it lies

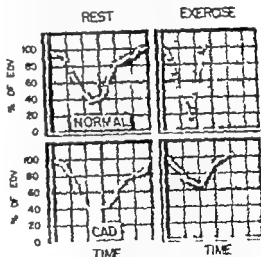


Figure 3 Effect of exercise on left ventricular function: left ventricular time-activity curves from studies shown in Figures 1 and 2. EDV denotes end-diastolic volume. Note that ejection fraction ($EDV - \text{end-systolic volume}$)

increases with exercise in the normal subject and decreases with exercise in the patient with coronary artery disease (CAD). (Figure and legend reprinted by permission from the New England Journal of Medicine 296: 842, 1977).

While the presence of coronary arterial narrowing which indicates regions of potential myocardial ischemia can be detected by coronary arteriography determination of the presence and severity of ischemia requires assessment of left ventricular function

However such assessments most commonly have been invasive, and have been associated with practical limitations which preclude routine or repeated evaluation and which most often have necessitated that studies be performed with the patient at rest. Since, even in the presence of relatively severe stable coronary artery disease left ventricular function is normal or near normal with the patient at rest an accurate indication of the presence and functional importance of ischemic heart disease requires study during stress such as that imposed on the heart by exercise.

Recently we have developed a real time radionuclide imaging system¹⁻³ which permits analysis of regional and global left ventricular function during intense exercise⁴. The technique involves acquisition with immediate display and analysis, of multi image electrocardiographically gated scintigrams in endless loop flicker free movie format. This imaging system has now been utilized in the study of more than 600 patients⁴⁻⁸ with vari-

ous forms of heart disease, and has proven particularly valuable in the detection of functionally important lesions of the coronary arteries.⁴⁻⁶

RADIONUCLIDE CINEANGIOGRAPHY METHODOLOGY

Gated cardiac scintigraphy is performed with subjects in the supine position during exercise. This procedure involves intravenous administration of a small amount of albumin labelled with 10 mCi of radioactive technetium (Tc^{99m}). After a period of 5 minutes to permit equilibration of the tracer in the blood pool a conventional Anger (field of view = 254 mm diameter) camera with a high sensitivity parallel hole collimator is oriented in the modified left oblique position¹⁻³ which permits imaging of the left ventricle in the field of view. Imaging then is accomplished at rest and exercise by the use of our computerized electrocardiographically gated procedure¹⁻³ modified to reduce data processing time and the interval required to achieve statistical reliability.^{3,4} The spatial resolution of this system is one centimeter.

When this procedure is employed, data is collected and concurrently organized

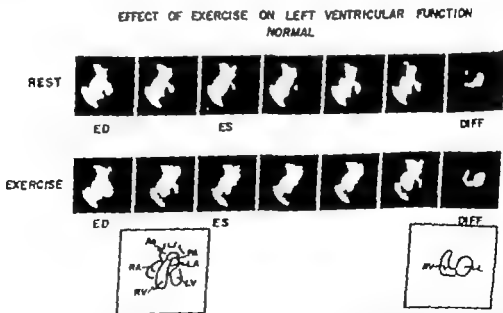


Figure 1 Unretouched selected frames in sequence from a radionuclide cineangiogram taken in a normal subject at rest (upper) and during maximal exercise (lower). ED denotes end-diastolic frame ES end-systolic frame DIFF difference image (created by electronic subtraction of end systolic counts from

end-diastolic counts) AO aorta PA pulmonary artery RA right atrium RV right ventricle LA left atrium and LV left ventricle (Figure and legend reprinted by permission from the New England Journal of Medicine 296:841, 1977)

OF STUDIES IN PATIENTS CORONARY ARTERY DISEASE

earliest studies demonstrated that, when performed during exercise the technique can be used to determine the presence and location angiographically demonstrable 150% of the coronary arteries even in patients whose regional and global left ventricular function are normal at rest⁴ (Table 1). In addition, these early studies demonstrated that in patients with coronary artery disease the response is directionally opposite to that of normal subjects, and that the ejection fraction developed during exercise in such patients is almost invariably below the range achieved by normal subjects (Figure 4). These results have been confirmed and extended in subsequent studies of larger groups of patients and normal subjects.^{2,6} These latter studies have demonstrated that the technique permits assessment of the degree of dysfunction in a given region, thus aiding in the elucidation of areas of potentially critically ischemic myocardium.² Such abnormalities of regional function are themselves diagnostic of is-

chemic heart disease since they are seen only in association with lesions in vessels supplying the abnormally functioning regions and are not seen in normal subjects or in regions supplied by normal arteries in patients with other stenotic vessels. Moreover regional abnormalities have been found during exercise even at levels of exertion not associated with symptoms.² Thus, in one study all 47 patients with coronary artery disease manifested regional dysfunction during exercise though 14 did not develop angina pectoris during the exercise study. Moreover ejection fraction in these 47 patients decreased from 48% at rest to 36% during exercise ($p < .001$) while in 25 age-matched normal subjects, ejection fraction invariably increased with exercise (av 57% at rest, 71% during exercise, $p < .001$), and no regional dysfunction was noted.² It is not surprising, therefore, that we have found radionuclide cineangiography during exercise to be more sensitive and more predictive than exercise electrocardiography in the diagnosis of coronary artery disease.⁶

Our studies also have demonstrated the utility of the technique in assessing the effect of therapeutic intervention.^{2,10} Thus, the sublingual administration of nitroglycerin has been shown to improve regional and global left ventricular function not only at rest but also and quantitatively more impressively during intense exercise, when ischemia would be most likely to be present.² Thus, in 28 patients given nitroglycerin ejection fraction during exercise before drug administration averaged 36% average ejection fraction rose to 48% during exercise after nitroglycerin. It is of interest that ejection fraction during exercise in normal subjects was unaffected by nitroglycerin. Moreover the beneficial effects of nitroglycerin were manifest even in patients who did not develop chest pain with exertion.² Similarly in many patients radionuclide cineangiography after coronary artery bypass grafting has demonstrated alleviation of regional and global dysfunction encountered during exercise prior to operation though no change has been noted in left ventricular function at rest in comparing pre and post-operative assessments.¹⁰

CLINICAL IMPLICATIONS

Stenoses of the coronary arteries can produce clinically important imbalance between myo-

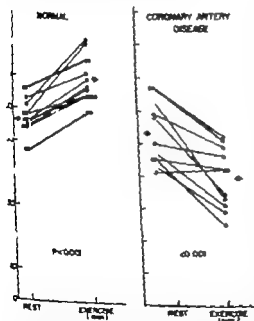


FIGURE 4 Effect of exercise on left ventricular systolic function showing the change in ejection fraction from rest to maximal exercise in normal subjects and patients with coronary artery disease (○ represents mean ejection fraction for entire group). (Figure and legend reprinted, by permission, from the *England Journal of Medicine* 296: 842, 1977).

within a physician selected temporal beat length "window." Distortion of the time activity curve by premature beats is prevented by rejection of cycles falling outside this window. The movie can be similarly windowed. Ejection fractions obtained by this method show excellent correlation with those obtained by contrast angiography with the patient at rest ($r=0.92$, $p<0.01$).⁹

After the images and time activity curves are obtained at rest, the subjects begin to pedal a bicycle ergometer. In the studies to be described imaging was continued for at least two minutes, until development of fatigue or typical angina of severity customarily causing the patient to stop exercising.

After completion of imaging, left ventricular ejection fractions at rest and during exercise automatically are determined from the computer-generated time-activity curves. Regional left ventricular function, at rest and during exercise, is determined visually from movies.

Since movies most often are constructed from images obtained in the modified left anterior oblique position, function of only

the anterosseptal and anterolateral walls can be evaluated from the movie. Assessment of other surfaces of the heart can be achieved by creating movies from images obtained with the camera oriented in a position other than modified left anterior oblique; however, to assess those regions which do lie on the edges of the cardiac silhouette in left anterior oblique position, our standard approach involves analysis of count-based "difference images" created by the end-systolic image from the end-diastolic image² (Figure 1-2). In the resulting difference image the intensity (brightness) of a region of the image is proportional to the absolute change in radioactive emission (volume) between diastole and systole in that region. In the left anterior oblique view a non-edge-bordered defect (that is, a region of darkness located centrally in the image and surrounded by bright regions) necessarily indicates the presence of either a posterobasal or anterobasal region from which blood is not being ejected (i.e., is not being ejected in as great a quantity as from surrounding regions).

Table 1

Correlation between Coronary Arteriographic and Exercise Radionuclide Cineangiographic Abnormalities.*

Case No.	Age (Yr)	FC NYHA †	Coronary Arteriographic Analysis ‡			Exercise Radionuclide Cineangiogram Analysis		Exercise Difference Image Analysis (Non Edge Bordered Defect)
			LAD	LCC	RCA	Antero-septal dysfunction	Antero-lateral dysfunction	
1	49	II	+	+		+	+	+
2	37	II	+			+		
3	62	III	+§	+§	+	+	+	+
4	51	III	+	+	+	+	+	+
5	50	II	+	+	+	+	+	
6	56	II	+	+	+	+	+	
7	52	III	+	+	+	+	+	+
8	40	II	+		+	+		
9	68	IV	+	+	+	+	+	
10	40	II	+			+		
11	50	I	+		+	+		+

+ denotes abnormality present & no entry abnormality absent. † New York Heart Association Functional Class.

‡ Arteries with >50% occlusions. LAD denotes left anterior descending coronary artery, LCC left circumflex coronary artery & RCA right coronary artery §Left main coronary artery >50% occlusion, in addition to LAD & LCC.

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SULTS OF STUDIES IN PATIENTS WITH CORONARY ARTERY DISEASE

earliest studies demonstrated that, when applied during exercise, the technique can be used to determine the presence and location angiographically demonstrable 150% doses of the coronary arteries even in patients whose regional and global left ventricular function are normal at rest (Table I). In addition, these early studies demonstrated that in patients with coronary artery disease the response is directionally opposite to that of normal subjects, and that the ejection fraction developed during exercise in such patients is almost invariably below the range achieved by normal subjects (Figure 4). These results have been confirmed and extended in subsequent studies of larger groups of patients and normal subjects.^{2,6} These latter studies have demonstrated that the technique permits assessment of the degree of dysfunction in a given region, thus aiding in the elucidation of areas of potentially critically severe ischemia.³ Such abnormalities of regional function are themselves diagnostic of is-

chemic heart disease since they are seen only in association with lesions in vessels supplying the abnormally functioning regions and are not seen in normal subjects or in regions supplied by normal arteries in patients with other stenotic vessels. Moreover regional abnormalities have been found during exercise even at levels of exertion not associated with symptoms.³ Thus, in one study all 47 patients with coronary artery disease manifested regional dysfunction during exercise, though 14 did not develop angina pectoris during the exercise study. Moreover ejection fraction in these 47 patients decreased from 48% at rest to 36% during exercise ($p < 0.01$) while in 25 age matched normal subjects, ejection fraction invariably increased with exercise (av 57% at rest, 71% during exercise $p < 0.01$) and no regional dysfunction was noted.³ It is not surprising, therefore, that we have found radionuclide cineangiography during exercise to be more sensitive and more predictive than exercise electrocardiography in the diagnosis of coronary artery disease.⁴

Our studies also have demonstrated the utility of the technique in assessing the effect of therapeutic intervention.^{2,10} Thus, the sublingual administration of nitroglycerin has been shown to improve regional and global left ventricular function not only at rest but also and quantitatively more impressively during intense exercise when ischemia would be most likely to be present.³ Thus, in 28 patients given nitroglycerin, ejection fraction during exercise before drug administration averaged 36% average ejection fraction rose to 48% during exercise after nitroglycerin. It is of interest that ejection fraction during exercise in normal subjects was unaffected by nitroglycerin. Moreover the beneficial effects of nitroglycerin were manifest even in patients who did not develop chest pain with exertion.³ Similarly in many patients radionuclide cineangiography after coronary artery bypass grafting has demonstrated alleviation of regional and global dysfunction encountered during exercise prior to operation though no change has been noted in left ventricular function at rest in comparing pre and post-operative assessments.¹¹

CLINICAL IMPLICATIONS

Stenoses of the coronary arteries can produce clinically important imbalance between myo-

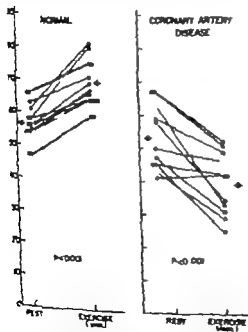


Figure 4. Effect of exercise on left ventricular systolic ejection fraction showing the change in ejection fraction on rest to maximal exercise in normal subjects and patients with coronary artery disease. (+) represents mean ejection fraction for entire group. (From and legend reprinted by permission from the New England Journal of Medicine 296:842, 1977).

cardial oxygen supply and demand. However such abnormalities most commonly are present only during stress when stenotic vessels are incapable of permitting augmentation of flow to maintain myocardial oxygen supply at a level sufficient to meet increased demand. Radionuclide cineangiography performed during the physiologic stress of exercise permits determination of the reserve capacity of the coronary circulation to meet such increasing demands and thus provides an accurate non invasive means of evaluating the functional importance of coronary artery lesions. The technique is effective in the detection of coronary artery disease and in the evaluation of the efficacy of therapy and would be expected to prove of value in the determination of prognosis.

It should be emphasized that while abnormalities of regional function are seen only in areas supplied by coronary arteries with $\geq 50\%$ stenoses, it is not true that all regions supplied by diseased arteries function abnormally nor that similar degrees of dysfunction are seen in all regions supplied by arteries with angiographically similar degrees of stenosis. Such variation in regional function might be attributable to variations in collateral supply or to variation in the actual degree of cross-sectional narrowing of vessels with angiographically similar stenoses.⁴ Because it provides a functional assessment of the importance of any coronary artery lesion and because it permits the physician to distinguish the relative functional importance of anatomically similar coronary artery lesions radionuclide cineangiographic assessment might prove a particularly useful method in the identification of those lesions which are potentially critical and which might be most likely to be associated with sudden death.

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**THALLIUM²⁰¹ EXERCISE TESTING
IN CORONARY ARTERY DISEASE**

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ABSTRACT

Myocardial imaging was performed following intravenous administration of Thallium²⁰¹ (Tl²⁰¹) at rest and during maximal exercise testing in 39 patients presenting with chest pain. Image data were computer analysed and compared with the results of coronary arteriography and left ventriculography.

In 26 patients with coronary artery disease (CAD) Tl²⁰¹ perfusion defects were present in 12 at rest and 24 on exercise. In 13 patients with normal vessels, perfusion defects were present in 2 at rest and one on exercise.

Resting Tl²⁰¹ image data corresponded to the presence or absence of impaired LV wall motion in 21 of 26 patients with coronary artery disease. Exercise scans were more accurate than rest scans in detecting the presence of CAD (Tl²⁰¹ scintigram—rest sensitivity—46 % exercise sensitivity—92 %, $P < 0.005$). Perfusion defects on the exercise Tl²⁰¹ scintigram tended to reflect the distribution of vessel disease, but prediction of individual coronary lesions from the scan findings was not possible.

These data suggest that Tl²⁰¹ myocardial imaging is a useful non-invasive technique for the investigation of patients presenting with chest pain. Rest Tl²⁰¹ scintigrams correspond to LV appearance on the ventriculogram and perfusion defects on the exercise Tl²⁰¹ scintigram detect the presence of CAD.

Radionuclide myocardial imaging provides a non-invasive technique for the investigation of patients with suspected coronary artery disease. Regional myocardial perfusion can be evaluated by imaging with a variety of agents (K^{43} , Rb^{81} , Cs^{137}) (2, 3, 5, 10, 16, 19, 20, 26). Thallium²⁰¹ (Tl²⁰¹) has physical and biological advantages over other currently available tracers (4, 13) and Tl²⁰¹ scanning is proving useful in the detection of myocardial infarction (9, 17, 23, 24, 26) & stress induced ischaemia (1, 11, 15, 21).

This study was designed to assess Tl²⁰¹ perfusion scintigraphy in predicting the presence or absence of coronary artery disease in patients presenting with chest pain. Scintiscans were obtained at rest and following exercise testing. Selective coronary arteriography and left ventriculography were performed in all patients and the results of the

radionuclide and angiographic studies compared.

PATIENTS AND METHODS

39 patients presenting with a history of pain sufficiently disabling to merit arteriography were studied. 37 patients: male 2 female; mean age 46.7 years \pm (mean \pm SEM) range 27–57 years. Scintiscans were obtained at rest and following exercise testing with an interval of at least 5 days between rest and exercise study. Both rest and exercise studies were within 14 days of cardiac catheterisation. The experimental nature of the study was explained to each patient and informed consent obtained.

Radionuclide Studies

Thallium²⁰¹ scintiscans were obtained at rest and following symptom limited maximal exercise testing.

Exercise Testing

Continuous multistage exercise testing was performed using a graded bicycle with initial work load of 300 KPM/min in increments of 300 KPM/min at 3 intervals. The electrocardiogram (modified electrode) was monitored continuously with sampling at 3 intervals and at the end of exercise. The tests were discontinued if chest pain (94 %) or dyspnoea (6 %).

The exercise electrocardiogram was interpreted independently without knowledge of the results of Tl²⁰¹ scan or angiography. A test was considered positive where there was horizontal or downsloping ST segment depression more than or equal to 1 m.m. at rest or after exercise.

Myocardial Imaging

Scintiscans were performed following intravenous injection of 2 mCi Thallium²⁰¹. Following a four hour fast, the rest injection was given in the upright position to minimise hepatic and gastric activity. The exercise injection was given at the onset of symptoms and the exercise terminated 30 seconds thereafter.

Myocardial imaging was performed using an Ohio Nuclear Series 100 Gamma Camera with a high resolution medium sensitivity p

left bore collimator. Scans were obtained in anterior 30° and 60° left anterior oblique and left lateral projections. The Gamma camera was interfaced to a Varian 620L computer and the data were stored on magnetic tape for later retrieval. Using the computer

interest were drawn corresponding anatomical areas of the left ventricle in the four scintigraphic projections which have been previously described by Morris and De Jong (25). The nuclide uptake in each of these areas was measured and computer analysis expressed as counts per area. In each view the area of maximal nuclide uptake was accepted as 100 % and the uptake in the remaining anatomical areas expressed as a percentage of this. In order to be the criteria of normal perfusions 15 healthy normal subjects were studied at rest and 10 after exercise testing.

In both rest and exercise scans, the uptake area to area varied by less than 15 %. Attention was paid to the apical area where visibly reduced uptake has been previously noted as a variant of normal (6). In this area, the count per unit area was less than 85 % of that in the area of maximal uptake. Thus, a perfusion defect was considered present in any area where this count per unit area was less than or equal to 80 % of the activity in the area of maximal nuclide

uptake. The coronary arteries were analysed. Patients with coronary artery disease were classified as having single, double or triple vessel disease according to the results of arteriography.

RESULTS

Radionuclide Studies

Satisfactory Tl^{201} scintiscans were obtained in all patients at rest and after exercise testing. Perfusion defects were present in the resting Tl^{201} scan in 14 patients and in the exercise Tl^{201} scan in 25 patients. In 20 patients, the distribution of the perfusion defects was more extensive on the exercise study than on the rest study.

Typical image data are represented in Figure 1. Panels a and c illustrate scans taken in this anterior projection after exercise and at rest respectively. Panels b and d illustrate scans from the same patient taken in the 30° LAO projection after exercise and at rest respectively. This patient was shown to have a normal left ventriculogram and an isolated > 50 % lesion of LAD. The rest studies (c and d) show homogeneous myocardial nuclide uptake. However both exercise studies (a

and b) show perfusion defects in the anterior and septal areas which were interpreted as indicating disease of the left anterior descending coronary artery. In the inferior area indicating right coronary artery disease. In the posterior and posterolateral areas as left circumflex artery disease.

Exercise Catheterisation

Left ventriculography and selective arteriography were performed by percutaneous femoral technique. Left wall motion was assessed visually from the right anterior oblique and left lateral projections at rest in the supine position. Abnormality was defined as any impairment of wall motion ranging from hypo- to dyskine-

tic. Significant coronary artery disease was defined as present in any vessel where luminal diameter was reduced by 50 % or more. For the purposes of this study the right coro-

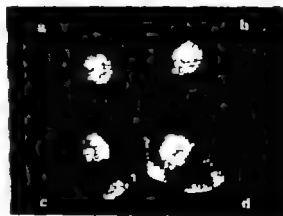


Figure 1. TYPICAL Tl^{201} IMAGE DATA. Scans obtained in a patient with normal L.V. wall motion and an isolated significant lesion of LAD. Panel a and c taken in anterior projection. Panel b and d taken in 30° LAO projection. Rest study—c and d exercise study a and b. Note homogeneous nuclide uptake in anatomical areas of L.V. in rest study. Perfusion defect with reduced nuclide uptake developed in the septal areas in exercise study corresponding to the disease of L.A.D.

ABSTRACT

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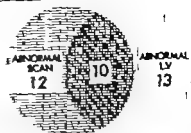
COMPARISON OF EXERCISE Tl²⁰¹ SCAN FINDINGS AND CORONARY ARTERY DISEASE IN 26 PATIENTS WITH SIGNIFICANT CAD

Vessel Disease	Exercise Tl ²⁰¹ Scan	
	Abnormal	Concordant with Distribution of Vessel Disease
single	6	6
double	13	14
triple	3	4

Exercise Tl²⁰¹ scan. However 2 patients with double vessel disease and 4 patients with triple vessel disease had an area of myocardium apparently normally perfused, but supplied by a significantly diseased artery. Two other patients (1 double 1 triple vessel disease) with extensive CAD had normal Tl²⁰¹ studies. In addition, although diseased vessels were usually associated with appropriate perfusion defects in the exercise Tl²⁰¹ scan, concordance was not reliable i.e. prediction of the individual diseased coronary artery by a location of underperfused areas was not accurate. This was particularly evident in RCA and LCx disease. 4 patients with disease of RCA, but not LCx had perfusion defects both inferiorly and posteriorly. Whilst, one patient with LCx, but not RCA disease had lateral i.e. inferior and posterior defects. Although the resting Tl²⁰¹ scan was an insensitive test in suggesting the presence of coronary artery disease, the resting Tl²⁰¹ scan findings tended to correspond to left ventricular wall motion seen in the ventriculogram (Fig. 2). Areas of impaired LV wall motion are present in 13 patients on the resting left ventriculogram (Table II). Perfusion defects are present in the resting Tl²⁰¹ scan in 12 patients. In 10 of these patients, the site of the perfusion defects seen in the scan corresponded to areas of impaired left ventricular wall motion on the ventriculogram. 3 patients, all with impaired inferior LV wall motion had normal rest Tl²⁰¹ scans whilst 2 patients with normal LV wall motion had perfusion defects (anterior septal + inferior and one antero-apical). 13 patients had normal left ventriculograms, 14 patients had normal rest Tl²⁰¹ scans and in 11 of these patients both the scan

RESTING SCAN

LV WALL MOTION



CONCORDANCE
21 of 26
81%

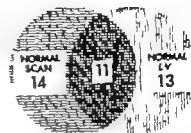


Figure 2 COMPARISON OF REST Tl²⁰¹ SCAN DATA AND LV WALL MOTION ON LEFT VENTRICULOGRAPHY IN 26 PATIENTS WITH SIGNIFICANT CORONARY ARTERY DISEASE. In 10 patients with perfusion defects on the rest Tl-201 scan the location of the perfusion abnormality corresponded to the site of impaired L.V. wall motion on the ventriculogram. In 11 patients note the Tl-201 scan at rest and the left ventriculograms were normal

and LV were normal. Overall, therefore the resting Tl²⁰¹ scan findings correspond to the presence or absence of impaired LV wall motion in 21 of 26 patients with coronary artery disease.

DISCUSSION

Increasing emphasis is being placed upon non-invasive techniques to identify patients with significant coronary artery disease. Myocardial imaging following the administration of radionuclide tracers provides a relatively non-invasive means of estimating regional myocardial perfusion (2, 3, 5, 10, 16, 19, 20, 26). Of the currently available tracers, Tl²⁰¹ appears to be one of the most promising (18), and Tl²⁰¹ scintigraphy is proving useful in detecting myocardial infarction (9, 17, 23, 24, 26) and stress induced ischaemia (1, 11, 15, 21).

This study demonstrates that image data from Tl²⁰¹ exercise scintigraphy can predict the presence or absence of coronary artery

and b) provided reduced nuclide uptake in the septal wedge corresponding to the significant disease of LAD

Angiographic Data

26 patients had significant coronary artery disease whilst 13 patients had normal coronary vessels or minor disease. 6 patients had single vessel disease (3 RCA 3 LAD) 15 double vessel disease (7 LAD + RCA 4 LAD + LCx 4 RCA + LCx) and 5 had triple vessel disease (LAD + RCA + LCx) (Table I) Isolated LCx disease was not encountered.

Table I

Category of Vessel Disease		Distribution of Disease
Single	6 patients	3 RCA 3 LAD
Double	15 patients	7 LAD + RCA 4 LAD + LCx 4 RCA + LCx
Triple	5 patients	LAD + LCx + RCA

RCA Right Coronary Artery

LAD- Left Anterior Descending Coronary Artery

LCx Left Circumflex Coronary Artery

In the 26 patients with coronary artery disease, LV wall motion on the resting left ventriculogram was normal in 13 and impaired in 13 patients. In 6 patients the anterior LV surface was involved in 3 patients the inferior surface and 4 patients the abnormality extended anteriorly to inferiorly (Table II)

Table II
LEFT VENTRICULOGRAPHIC FINDINGS IN 26 PATIENTS WITH SIGNIFICANT CAD

Left Ventriculogram		Number of patients
Normal Abnormal	Anterior Surface	6
	Inferior surface	3
	Anterior Inferior Surface	4
		13

Comparison of Data

In the 26 patients with significant coronary artery disease perfusion defects were present in the resting Tl^{201} scan in 12 patients and in the exercise Tl^{201} scan in 24 patients (92%) (Table III) Perfusion defects indicating the presence of CAD were significantly

Table III
 Tl^{201} SCAN FINDINGS IN 26 PATIENTS WITH CORONARY ARTERY DISEASE

	Abnormal Scan	N Scan
Tl^{201} Scan—Rest	12 (46 %)	14
Tl^{201} Scan—Exercise	24 (92 %)	2

* $p < 0.005$ — Perfusion defects significantly more frequent in exercise scan compared to rest

more frequent in the exercise scan than the resting scan (24 patients—Exercise scan patients—Rest scan $p < 0.005$). 2 patients with triple vessel disease and one with disease of RCA + LAD had normal Tl^{201} scans both at rest and exercise.

In 13 patients with normal coronaries, minor disease the Tl^{201} scan was normal in 11 patients at rest and in 12 patients at exercise (Table IV) 2 patients with normal coronary arteries had perfusion defects anterior in the resting Tl^{201} scan one of which was persistently present in the exercise Tl^{201} scan. Both patients had abnormal exercise ECG.

6 patients had single, 15 double and 5 vessel disease. In 18 of those 26 patients perfusion defects in the Tl^{201} exercise scan were located in anatomical areas which corresponded to vessel disease (Table V). All 6 patients with single vessel disease and 12 of the 15 patients with double vessel disease had appropriately sited underperfused areas

Table IV
 Tl^{201} SCAN FINDINGS IN 13 PATIENTS WITH NORMAL CORONARY ARTERIES MINOR CORONARY DISEASE (< 50% OCCLUSION)

	Normal Scan	Abnormal Scan
Tl^{201} Scan—Rest	11	2
Tl^{201} Scan—Exercise	12	1

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disease in patients presenting with chest pain. In 26 patients with arteriographically documented CAD 24 (92 %) demonstrated Tl^{201} perfusion defects after symptom limited exercise testing. 2 patients, one with double and one with triple vessel disease had normal Tl^{201} scans both at rest and exercise. There were no apparent features to identify those patients with false negative tests from those in whom perfusion defects developed. The high sensitivity in this study compared to other published data (1 15 21) may be in part, related to this study group since only a small proportion (5 of 20 patients) had triple vessel disease where failure to see a decrease in focal tracer uptake might be expected (18). The semi quantitative analysis of regional nuclide uptake may be another factor since this tended to reduce observer variation. However this technique itself introduced inherent limitations since quantification of regional perfusion can only be relative and not absolute. The nuclide uptake in each anatomical area was compared to the zone with maximal uptake. This zone may have been better perfused than other areas but may well have been supplied by a significantly stenosed coronary vessel.

The group of patients with normal coronary arteries or minor disease is small predominantly male and since the presenting symptom in each case was chest pain can scarcely be classed as "normal". Nevertheless, perfusion defects in Tl^{201} exercise scintigraphy proved specific for the presence of CAD in all but one patient. This patient had a history of a typical chest pain a positive exercise test and an anterosseptal image defect both at rest and on exercise and may represent one of the group of patients with "angina" but normal coronary arteries (12, 14). Image data from the resting Tl^{201} scan was less useful in predicting the presence or absence of CAD. However blood flow and regional distribution abnormalities due to coronary artery stenosis of 50 % or more may only become apparent during exercise or pharmacological challenge (7 8). Hence since myocardial Tl^{201} distribution parallels flow (22) Tl^{201} scintigrams at rest may not detect haemodynamically significant CAD.

The findings on the resting Tl^{201} scan corresponded to LV wall motion on the ventriculogram. 10 of the 12 patients with perfusion

defects at rest had corresponding areas of impaired LV wall motion on the resting ventriculogram whilst 11 of the 13 patients with normal LV wall motion but significant CAD had normal rest Tl^{201} scans. This supports the conclusion from other data that resting Tl^{201} perfusion defects represent cardiac infarction (9 17 23-24 26).

The exercise Tl^{201} scintigram was useful in detecting the presence or absence of coronary artery disease and the site of a perfusion defect corresponded to a diseased vessel. However prediction of individual coronary stenosis from the exercise image was not possible. Regions supplied by vessels angiographically documented disease in some instances did not develop perfusion defects, particularly in patients with triple vessel disease. In others, the image defect could imply either RCA or LCx disease, or both. Several explanations can be proposed. 1. anatomical distribution of vessels, whether right or left dominant, or balanced. 2. lateral and the contribution of collateral circulation may have affected the nuclide uptake. The development of ischaemia in one of a number of potentially ischaemic zones may have produced a Tl^{201} scan underestimating the extent of disease. As indicated above, the technique of analysis has limitations which will influence the final prediction of individual coronary lesions.

In conclusion these results indicate that Tl^{201} imaging was a useful non invasive technique in the investigation of patients with suspected coronary artery disease. Perfusion images after exercise were more sensitive in detecting coronary artery disease than the rest image. The resting image was useful to the presence or absence of impaired LV wall motion. Perfusion defects on the exercise Tl^{201} scan tended to correspond to the distribution of vessel disease but prediction of individual coronary disease from the exercise findings was not possible.

11.

EFFECT OF DRUGS ON MYOCARDIAL PERFUSION SCANNING AND WALL MOTION IN PATIENTS WITH CORONARY INSUFFICIENCY

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**EFFECT OF DRUGS
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WALL MOTION IN PATIENTS WITH
CORONARY INSUFFICIENCY**

**H Simon,
R. Felix,
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The aim of any antianginal therapy is to relieve ischemia and possibly to restore compromised ventricular function. We therefore investigated the effect of oxyfedrin, a positively inotropic acting drug, a betablocking substance (Bupranolol) and a nitro compound on regional blood flow, regional blood flow distribution and regional wall movement in a poststenotic asynergic myocardial region in patients with coronary heart disease. We were interested to see the effect of the antianginal drugs on this poststenotic region and whether there was a correlation between local poststenotic blood flow and local wall movement.

METHODS

In 30 patients with the clinical diagnosis of coronary insufficiency who were subjected to coronary arteriography and laevocardiography the local poststenotic blood flow in the asynergic region was measured by the ^{133}Xe washout technique by means of a scintillation counter which was firmly placed over the chest when its location corresponded to the distal part of the left anterior ventricular wall. The diameter of the collimator was 2.9 cm. The blood flow distribution was obtained by slow injection of radioactive labelled microspheres (500 nCi ^{133}Xe , 5 mCi $^{99\text{m}}\text{Tc}$) into the left coronary artery. The scan was done one hour after the investigation in right oblique projection by means of a Gamma camera. (1) Care was taken to collect as many counts as possible (ca 700 000 per projection) in order to get a good spatial resolution and scinti photo. The distribution of perfusion in the asynergic region was evaluated as the percentage of radioactivity in this myocardial region as compared to the point of maximum activity. Local wall movement out of the silhouette of the laevo was determined by drawing a short axis R^{A} perpendicular to the longitudinal axis of the left ventricle in the distal third of the left anterior ventricular wall in the asynergic region in RAP projection. The amount of the systolic shortening of this half axis R^{A} was taken as an index of local wall movement in the poststenotic region.

The following hemodynamic data were recorded: Heart rate, left ventricular enddiastolic pressure, mean pressure in the aorta, ejection fraction and circumferential fibre

shortening velocity. The substances were oxyfedrin ($n=10$), Bupranolol ($n=10$) and Isosorbiddinitrat, a long acting nitro compound ($n=10$). The investigation carried out in the following way:

1. Recording of hemodynamic data, flow (^{133}Xe) blood flow distribution, wall movement (laevocardiogram).
2. Application of the drug (8 mg oxyfedrin i.v., 8 mg Bupranolol i.v., 40 mg Iso-retard forte p.o.)
3. Repeating of the above measurements after a definite hemodynamic effect of the drug could be observed.

RESULTS

1. The coronary arteriogram showed the following findings:
 - a) Oxyfedrin group ($n=10$): 5 patients had more than 50% stenosis of the anterior descending artery (LAD), 3 times before the branching off of septal branch (proximal), twice distal. In the other 5 patients the stenosis was less than 50% and four times located proximal to the first septal branch and once distal.
 - b) Isosorbiddinitrat group ($n=10$): In seven patients there was a proximal and one patient a distal stenosis of the LAD more than 50% up to 90% whereas in three patients the stenosis was less than 50%.
 - c) Bupranolol group: Seven patients had a proximal stenosis of the LAD of less than 50% and up to 90% while in three patients the lesions were less than 50%.
2. The hemodynamic data before and after the application of the substances are summarized in Table I.
3. Local blood flow: The regional myocardial blood flow in the poststenotic region increased significantly in 7 out of 10 patients under the positively inotropic action of oxyfedrin while no change or only a minimal rise was observed in 3 patients. (1). Regional flow increased in 5 out of 10 patients under isosorbiddinitrat. A decrease was observed twice and no change in 3 patients. Under betablockade a decrease in local flow was seen in 6 patients, a rise in 2, no change in 3 patients. Differences between the groups were

My I
 hemodynamic data (HR = Heart rate, LVEDP = Left ventricular enddiastolic pressure, AOP = Mean
 ic pressure, EF = Ejection fraction, V_{CF} = Circumferential fibre shortening velocity), local
 stenotic flow and local wall motion in the poststenotic asynergic region (R^A) before and after 8 mg
 yfedrin, 8 mg Bupranolol iv and 40 mg Iso-Mack retard forte p.o. The values given are the mean
 se and the standard deviation of the mean value (S_E).

	Oxyfedrin		Isosorbiddinitrat		Bupranolol	
	Before	After	Before	After	Before	After
time)	70.0 ± 2.9	77.6 ± 3.9	80.4 ± 4.4	77.4 ± 2.6	81.3 ± 2.4	71.0 ± 1.8
p	< 0.05		n.s.		< 0.001	
EDP mm Hg)	13.6 ± 1.6	18.9 ± 1.4	20.4 ± 1.4	11.8 ± 1.5	12.3 ± 2.0	15.1 ± 1.4
p	< 0.01		< 0.001		< 0.01	
DP mm Hg)	100.8 ± 5.3	104.9 ± 6.6	107.4 ± 6.7	96.6 ± 5.4	100.4 ± 3.6	102.5 ± 3.3
p	n.s.		< 0.05		n.s.	
)	57.4 ± 3.2	66.5 ± 4.8	—	—	62.5 ± 3.0	54.2 ± 3.9
p	n.s.		—		< 0.01	
7 mm/ s ⁻¹)	1.13 ± 0.09	1.48 ± 0.09	—	—	1.18 ± 0.18	0.95 ± 0.11
p	< 0.01		—		< 0.05	
ow 1/100g/ s)	38.3 ± 2.9	54.1 ± 6.4	52.8 ± 4.3	59.6 ± 2.7	55.8 ± 3.5	49.6 ± 4.1
p	< 0.05		n.s.		n.s.	
)	23.4 ± 3.3	39.2 ± 2.5	14.3 ± 4.0	28.9 ± 4.2	24.5 ± 2.2	15.6 ± 2.8
p	< 0.01		< 0.05		< 0.01	

ever not significant (Fig. 2) in either the
 nitro group or the betablocker group.
 Local blood flow distribution. A signifi-
 cant redistribution into the poststenotic
 region was seen in 6 patients under oxyfe-
 drin, (Fig. 3) in 5 under beta-receptor
 blockade and in 5 under isosorbiddinitrat.
 This redistribution was combined with an
 increase in local blood flow in 5 out of 6
 patients under oxyfedrin, in the 5 under

isosorbiddinitrat and in no patient under
 B-blockade.

- 4 Local wall motion. The poststenotic wall
 motion improved significantly in both the
 oxyfedrin and isosorbiddinitrat group
 with 7 and 4 patients respectively normal-
 izing the systolic shortening of the half
 axis in the asynergic region. On the con-
 trary in the betareceptor blocking group 8
 deteriorated and 2 stayed unchanged.

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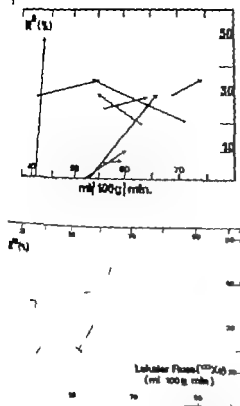


Fig. 4 Regional poststenotic blood flow (abscissa) and regional poststenotic wall motion (ordinate) before and after isosorbiddinitrat (4a) and before and after oxyfedrin (b).

noted in 2 patients under beta-blockade would be due to the special situation where the oxygen requiring effect of an increased ventricular diastolic wall tension is not offset by the oxygen saving effect of a decreased frequency and a reduced contractility (2). An increase in local blood flow and an improved wall motion was under isosorbiddinitrat and oxyfedrin usually combined with shift of blood flow into the poststenotic region which can be interpreted as recruitment of the coronary flow reserve within the viable myocardium in the poststenotic zone. It was only under beta-blockade where no correlation between flow and flow distribution existed. The finding of a redistribution of blood-flow into the poststenotic synergic region in 5 patients under beta-blockade is compatible with animal experiments in the chronically ischemic myocardium (4). It shows furthermore the additional information which can be gained by the perfusion scintigram. A parallelism between an

improved perfusion and an enhanced wall motion was above all seen under oxyfedrin when the increase in contractility induced a rise in blood flow. This parallelism was only present in half of the patients under isosorbiddinitrat and in no patient under beta-blockade. Under a nitrocompound the local wall motion seems to be dependent both on a decrease of preload and afterload (3) and on an increase in local blood flow (2) whereby the interaction between these 2 factors varies from patient to patient.

That most probably hemodynamic factors or the state of contractility play a greater role in the poststenotic wall motion than blood flow is underlined by the finding of an impaired local wall movement under beta-blockade despite a redistribution of blood flow into the poststenotic myocardial region in 5 patients or an increase in blood flow in another 2 cases.

SUMMARY

- 1 There are several groups of drugs which can be of use in patients with coronary heart disease.
- 2 The therapeutic effect of nitrocompounds is due to both a decrease in pre and after load as well as an increase in local poststenotic blood flow with a concomitant improvement in local wall movement in the synergic region. Beta-receptorblockers always increase the degree of synergy in the poststenotic region which in combination with a reduction of frequency and contractility is an oxygen saving effect.
- 3 The relation between regional poststenotic blood flow and wall motion under therapy is unpredictable and varies as much with different drugs as among patients.
- 4 Unless one has extensive, invasively obtained information on a patient, which, however is not feasible in most cases, the therapy is always a trial and error procedure. It is for this reason that it seems to be sensible to combine drugs of different action like nitro-compounds and beta-receptorblockers.
- 5 In some patients with coronary heart disease the therapeutic goal can be achieved not only by oxygen saving drugs but also by a positively inotropic acting substance.

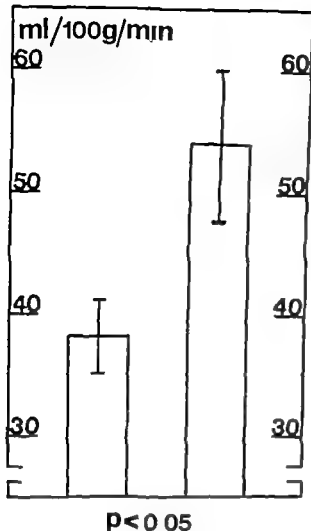


Fig 1 Regional poststenotic blood flow before (first column) and after (second column) 8 mg oxyfedrin i.v.



Fig 3 Regional poststenotic flow distribution before (3a) and after (3b) oxyfedrin. The perfusion area in the distal part of the left anterior ventricle (arrow) is almost abolished after the drug. Besides the size of the ventricle has decreased as consequence of the increased contractility under oxyfedrin. The scans were taken in anterior— projection.

- Local blood flow and local wall movement in the asynergic region. A parallelism between an increase in blood flow and improved wall movement was seen in the oxyfedrin group in 7 out of the patients and in the nitrocompound in half of the patients (Fig. 4). Under receptor blockade there was a striking parity between flow and wall motion decrease in flow being combined with change in the local contractile pattern or decrease in the systolic shortening during despite an increase in flow.

DISCUSSION

The results show that substances of different chemical structure can influence local blood flow in a poststenotic asynergic myocardial region. The most pronounced effect was under the positively inotropic acting oxyfedrin most probably as the result of an increase in contractility. The rise in flow was seen under isosorbiddinitrat was with one exception concomitant with a decrease in pre- and afterload whereby the decreased filling pressure in the left ventricle could enhance local flow via a diminished extravascular coronary resistance. The decrease in poststenotic blood flow which occurred under beta blockade can be explained by reduction in frequency and contractility. The rise in local blood flow however which occurred

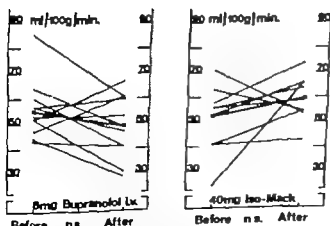


Fig 2 Regional poststenotic blood flow before and after Bupranolol (left side) and before and after Isosorbiddinitrat (right side)

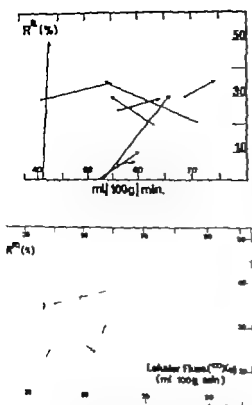


Fig. 4 Regional poststenotic blood flow (abscissa) and regional poststenotic wall motion (ordinate) before and after isosorbide dinitrate (4a) and before and after oxyfedrin (4b).

tered in 2 patients under beta-blockade could be due to the special situation where the oxygen requiring effect of an increased ventricular diastolic wall tension is not offset by the oxygen saving effect of a decreased frequency and a reduced contractility (2). An increase in local blood flow and an improved wall motion was under isosorbide dinitrate and oxyfedrin usually combined with shift of blood flow into the poststenotic region which can be interpreted as redistribution of the coronary flow reserve within the viable myocardium in the poststenotic region. It was only under beta-blockade where no correlation between flow and flow distribution existed. The finding of a redistribution of blood flow into the poststenotic synergic region in 3 patients under beta-blockade is compatible with animal experiments in the chronically ischemic myocardium (4). It shows furthermore the additional information which can be gained by the perfusion scintigram. A parallelism between an

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- 5 In some patients with coronary heart disease the therapeutic goal can be achieved not only by oxygen saving drugs but also by a positively inotropic acting substance.

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**MEXILETINE EFFECT
ON MONOPHASIC ACTION POTENTIAL
(MAP) OF RIGHT VENTRICLE IN MAN**

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In the analyses of single ventricular ectopic beats during acute myocardial infarction the interest has mainly been focused upon the time relation between the start of the ectopic beat and the repolarization of the preceding beat. However apparently identical premature ventricular ectopic beats may or may not result in repetitive ventricular dysrhythmias. Therefore the refractory state after the ectopic beat must be of equal interest as the excitation process of that beat.

We have therefore aimed to study the repolarization as well as the propagation of induced ectopic ventricular beats and the influence of Mexiletine, an antiarrhythmic drug with electrophysiological properties similar to Lignocaine (1, 2, 3, 10)

MATERIAL AND METHODS

The study was performed on 9 healthy male volunteers. It was presented for and approved by the Ethical Committee of the hospital.

By means of a percutaneously introduced disposable suction electrode catheter* the monophasic action potential (MAP) was recorded from the outflow tract of the right

ventricle (4). In addition a bipolar pacing catheter was placed at the apex of the ventricle for programmed ventricular pacing (5). The effective refractory period of the ventricle was determined via the pacemaker catheter with a precision of 2 msec. The cycle was then paced with a regular basic cycle length of 600 or 500 msec. Premature stimuli were thereafter introduced every 8th to 10th beat in such a way that basic cycle length was scanned with ectopic stimuli from the value of the effective ventricular refractory period up to the basic cycle length. The refractory period the premature stimuli were given with increments of 5 msec. MAP was recorded continuously during programmed ventricular pacing. The programmed ventricular pacing with MAP recording was then repeated in the same manner described above 10 min after a slow bolus injection of 2 mg Mexiletine/kg BW.

Precordial ECG, pacemaker signal, MAP and RV electrogram were recorded on tape. The records were then replayed and analysed with respect to the parameters demonstrated in fig. 1 and 2. The time from pacemaker stimulus of the last basic cycle beat to the onset of depolarization of the

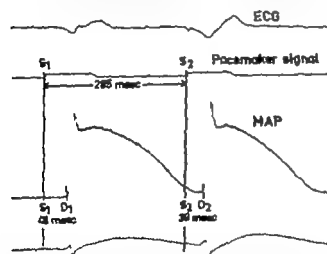


Fig. 1 Example of MAP recording and time interval calculation. From top to bottom: Precordial ECG, pacemaker signal, MAP signal, right ventricular electrogram.

S₁ = regular pacemaker stimulus, S₂ = premature pacemaker stimulus, D₁ = Start of depolarization of MAP during regular pacing, D₂ = Start of depolarization of MAP during ectopic beat.

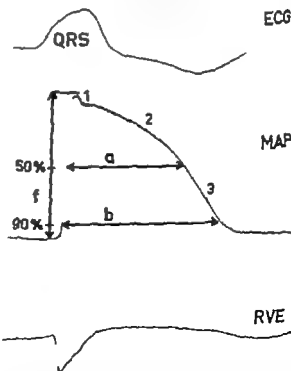


Fig. 2 Map recording from subject no. 7 to show the relationship between the MAP signal to ECG and right ventricular electrogram. The duration of MAP is indicated at the level of 50 and 90% repolarization.

ular MAP (S_1-D_1) C = basic conduction time) was measured as well as the conduction interval of the early ectopic beat (S_1-D_1). The measures were then related to a value of the basic cycle length (S_1-S_2) and the premature interval (S_2-S_3). In this way we analyzed the conduction of all premature beats as well as the conduction during regular pacing.

For assessment of repolarization during ectopic beats, the duration of the MAP was measured at the level of 90 % repolarization. The same analysis was also done from records during the regular pacing.

RESULTS

Conduction

The conduction time of the early ectopic beat, S_1-D_1 , is related to the prematurity of the ectopic impulse S_1-S_2 in a characteristic way (fig 3 and table I). Close to the refractory period, the time from right ventricular apical stimulation to outflow tract excitation is substantially longer than that during the basic paced beat. There is also a period when the conduction time is significantly shorter than that of the basic paced beat—phase of accelerated impulse propagation.

Table I
Effect of Mexiletine upon Conduction and Repolarization during Regular Pacing and during Ectopic beats (All Values in msec).

No.	Analyses during regular pacing		Analyses of earliest excited ectopic beat		MAP-duration at 90 % repolarization		Analyses of super normal ¹ beat	
	S_1-D_1 2 sd		$(S_1-D_1)_{max}$		MAP-duration at 90 % repolarization		$(S_2-D_2)_{min}$	
	C	M	C	M	C	M	C	M
39	4.0	46 2.1	44	64	198	208	32	46
57	3.4	71 2.2	68	92	196	200	50	64
67	2.3	81 2.4	82	118	174	185	56	78
48	1.8	51 2.0	50	60	180	190	38	50
72	4.0	80 3.0	106	116	188	198	64	70
81	4.4	89 3.6	106	116	200	212	72	86
79	2.8	79 2.4	96	96	176	210	74	76
37	2.4	45 3.1	36	50	200	218	32	42
Mean	59	66	71	85	194	205	51	62
Difference	$p < 0.01$		$p < 0.01$		$p < 0.01$		$p < 0.01$	

Abbreviations: C = control values before mexiletine. M = values after mexiletine.
Other abbreviations: see text.

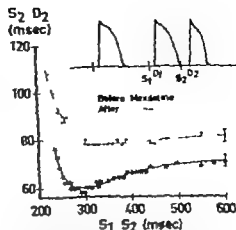


Fig 3 Effect of coupling interval and of Mexiletine upon conduction of ectopic impulses from pace-maker electrode in apical position in right ventricle to outflow tract of right ventricle

Repolarization

The duration of the MAP of the early ectopic beat is markedly affected by the prematurity of the beat, e.g. the preceding cycle length (fig 4 and table I). The duration measured at 90 % repolarization of beats close to the refractory period is approximately 40 msec shorter than that of the basic paced beat.

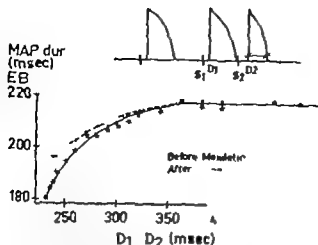


Fig 4 Effect of prematurity and of Mexiletine upon duration of MAP of early ectopic beats

Mexiletine

The conduction of the early ectopic beat is markedly influenced by the drug (fig 3 and table I). The accelerated propagation of the ectopic beat is almost completely abolished and the delay of the earliest ectopic beat is more pronounced than before the drug was given. In addition the basic conduction time is slightly prolonged. All these effects are statistically significant.

Repolarization is also affected by Mexiletine. The shortening of the MAP of the early beats is thus much less pronounced than before the drug was given (fig 4 and table I). Again this is highly significant. The difference loses its significance when the $D_1 - D_2$ value amounts to more than 50 msec, over the value of the refractory period. During regular pacing the MAP duration is unaffected by the drug.

DISCUSSION

The present investigation has demonstrated several findings that may be of importance for the antiarrhythmic action of Mexiletine. The conduction of the impulse from the apex to the outflow tract of the right ventricle is delayed during regular rhythm. This decreased conduction velocity is likely to be due to the decreased maximal rate of rise of depolarization of myocardial cells caused for Mexiletine (3). This influence upon maximal depolarization rate is an effect noted for several antiarrhythmic drugs (6).

The rate of rise of an action potential is strictly dependent upon the actual membrane

polarization (7). Therefore the rate of an action potential will be decreased elicited during the repolarization phase preceding action potential. This is probably the explanation why the conduction of the impulses close to the refractory period is prolonged compared with that of the paced beat. The mechanism of this conduction delay has been more extensively discussed elsewhere (8). The exaggeration of conduction impairment of early ectopic beats caused by Mexiletine may be another of importance for its antiarrhythmic action. This has been more extensively discussed elsewhere (8).

The influence upon the supranormal phase conduction of ectopic beats is pronounced than the effect on the conduction of the very early ectopic beats. A probable electrophysiological explanation for the normal phase is that the signal passes through cells with early phase 4, e.g. Purkinje cells. As these have a high brane polarization at the moment of initiation, the rate of rise of the action potential will be increased and thereby also most likely the conduction velocity (9). The effect of Mexiletine has been studied upon the action potentials of the cells of the specialized conductive system that most likely limit the refractory period in the way estimated in this study (3). It is, however, difficult to explain the influence of Mexiletine upon the supranormal conduction from these studies. A slight action potential shortening has been observed elsewhere (3). The effect of Mexiletine upon the repolarization of the early ectopic beats is, however, a new finding. No effect has this effect been described in literature, although an effect of any other antiarrhythmic drug has perhaps due to the fact that enough attention has not been focused upon the repolarization of early ectopic beats.

Another class of antiarrhythmic drugs, however, delay ventricular muscle repolarization already during regular rhythm (6). It is therefore tempting to believe that even this effect may play a role in the antiarrhythmic mechanism of Mexiletine.

Conduction and repolarization-effects of Mexiletine upon early ectopic beats become more pronounced because of a delay of repolarization after early ectopic impulses. The time after an early ectopic impulse when an apart from supranormal

Part of the myocardium reaches excitability—
and thereby vulnerability—will thus be sub-
stantially delayed by Mexiletine. It may be
speculated that these effects are active also
upon concealed impulses, thereby facilitating
the antiarrhythmic activity of Mexiletine.

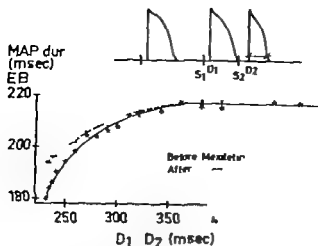


Fig 4 Effect of prematurity and of Mexiletine upon duration of MAP of early ectopic beats

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Another class of antiarrhythmic drugs do however delay ventricular muscle repolarization already during regular rhythm (6). It is therefore tempting to believe that even this effect may play a role in the anti arrhythmic mechanism of Mexiletine.

Conduction and repolarization-effects of Mexiletine upon early ectopic beats both cause a delay of repolarization after early ectopic impulses. The time after an early ectopic impulse when an apart from stimulus

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Survey of Serum Lipid Levels in Icelandic Men Aged 34-61 Years

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By

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Ólafur Ólafsson

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Þorsteinn Þorsteinsson

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originally published as *Nordiskt Medicinskt Arkiv* was founded in 1869 by Professor Axel Key MD. In 1901 (from volume 34) this journal was divided into a medical and a surgical section. Since 1919 (from volume 52) the medical section has been published under the name of *Acta Medica Scandinavica*.

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FOREWORD

This report from the Heart Preventive Clinic of the Icelandic Heart Association is one in a series of reports on a prospective health survey conducted in the Reykjavik area since 1967

The emphasis in these reports is on the publication of results and their chief statistical characteristics in the form of computer printed tables and graphs

They include detailed descriptions of the methods used in the health survey as well as various accompanying documents Discussion and references to other work is limited as the purpose of the report is primarily to publish detailed results in an accessible format

This report deals with a matter of special interest in cardiovascular epidemiology the blood lipids

Tables and figures in reports on the analysis of data from the Icelandic Heart Association's Health Survey in the Reykjavik area are labelled with a capital letter which refers to the stage of the survey a Roman numeral referring to the number of the report and an Arabic number which is the number of the table or figure in that report Thus Table A III 1 is the first table in the third report on the first stage of the survey

Thanks are due to Mr H Filippusson Ph D Dept of Clin Chem University Hospital Reykjavik for the translation of this report into English

We also acknowledge gratefully financial support from The Debt Collection Fund of Icelandic Banks which made this publication possible

Reykjavik October 1977

The Report Committee

HEALTH SURVEY IN THE REYKJAVIK AREA 1967 - ■■

INTRODUCTION

In the autumn of 1967 the Icelandic Heart Association embarked on Stage I of an extensive health survey. Approximately one-third of each of 16 age groups of men aged 34 to 61 years domiciled in the Reykjavik area were invited to participate in Stage I which was almost completed by the autumn of 1968 [1].

The primary aim of the health survey which is a prospective study is

- 1) to detect cardiovascular and various other diseases in their early stages to find the prevalence and incidence of these diseases as well as their causes in search of preventive measures
- 2) to assess the financial and health benefits to be gained from such mass screening programs

To achieve these aims Stage I of the male survey included a study by means of a questionnaire of the medical and social history of the participants heart and lung X-ray spirometry electrocardiography phonocardiography measurement of blood pressure height and weight as well as roentgenograms on bones and skin fold thickness a glucose tolerance test blood and urine analysis and a medical examination

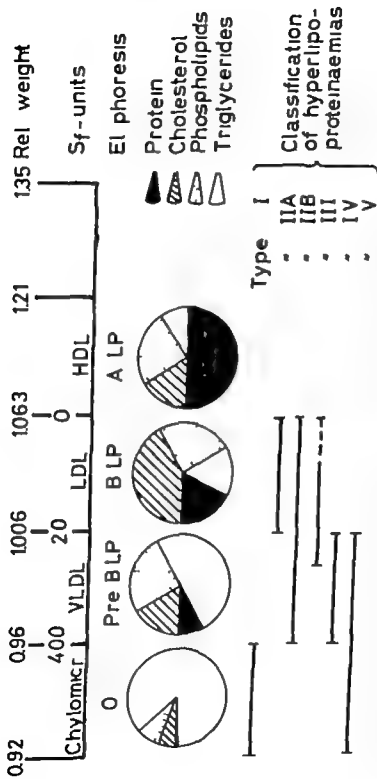



FIGURE A III.1. Top: Separation of a true lipoprotein by ultracentrifugation and electrophoresis. Bottom: Classification of hyperlipoproteinaemias according to Fredrickson and Le

Diseases of the coronary and other arteries often occur with other diseases such as diabetes diseases of the kidney diseases of the thyroid and others. Elevated concentrations of cholesterol and various other lipids commonly occur with these diseases as well as fatty deposits in the walls of blood vessels and elsewhere in the body.

Chemical analyses of fatty deposits in the walls of blood vessels from patients with the diseases referred to above have shown them to be rich in cholesterol as well as other lipids showing various stages of calcification.

Most of the cholesterol in blood is together with phospholipids and triglycerides bound to proteins in so called lipoproteins.

Ultracentrifugation can be employed to classify lipoproteins according to their sedimentation behaviour which is measured in so called Svedberg flotation units (S_f). The different subclasses are identified by their respective S_f values.

Lipoproteins have also been classified according to their electrophoretic mobilities. Thus the lipoproteins have been divided into the following main classes: Chylomicrons, pre- β lipoproteins, β lipoproteins and α lipoproteins. By methods based on this principle various types of hyperlipoproteinaemias have been identified. Fig. 1 shows the classification of lipoproteins according to the above mentioned methods and their interrelationships as well as the major types of hyperlipoproteinaemias characterised by elevation of the various classes of blood lipoproteins. 

Recent studies have indicated that for males under the age of 50 years there is a closer association of coronary diseases with elevated cholesterol β lipoproteins (S_f 20-400) than with elevated α lipoproteins (S_f 0-12). [3] [4]

Until the blood lipids cholesterol has been most thoroughly investigated since it is most easily determined whereas until recently only very few laboratories had at their disposal the

techniques necessary to thoroughly study the various other lipids

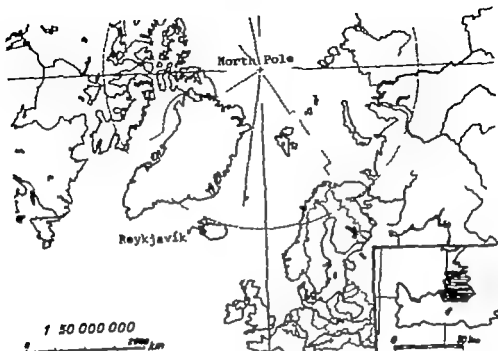
Even though it is considered proven that high concentrations of cholesterol and various other lipids in serum are risk factors the problem of the biological connector remains unsolved

In addition to much experimentation with animals a large number of health surveys have been carried out in the last two decades in order to throw light on this problem. In the United States and in various European countries these have been follow-up studies aimed among other things at investigating the development of cardiovascular diseases in people with different living habits. These studies have cast some doubt on the importance of various known risk factors for cardiovascular diseases such as cigarette smoking and body fatness [5]

These studies will not be further elaborated on here except to point out that health surveys show up variations from one nation to another in the numerical characteristics for serum cholesterol concentration. The results of surveys carried out in other countries cannot lead to any definite conclusions about the concentration of cholesterol in the serum of Icelanders [6]

No systematic investigations of lipids in the human body have been carried out in this country except for a study of the effect of cod liver oil administration on serum cholesterol concentration in elderly people. That study did however indicate that Icelanders were among those nations with the highest cholesterol concentration [7]

The subject of this report is the measurement of serum concentrations of β lipoproteins, cholesterol and triglycerides. The main emphasis is on the distribution of these values and their main statistical characteristics, e.g. mean values, standard deviations and several fractiles.



METHODS

Population.

Iceland is a volcanic island in the North Atlantic Ocean close to the Arctic circle with an area of 103 000 sq km.

In the years 1931-60 the mean monthly temperature in Reykjavik varied between -3.8°C to $+3.2^{\circ}\text{C}$ in the coldest month January and between 10.1°C to 13.2°C in the warmest July. The mean annual number of rainy days ($>1.0\text{ mm}$) was 212. The mean annual number of hours with bright sunshine was in June 189 and 8 in December [8].

According to historical documents, settlement was established in the 9th and 10th centuries AD mainly from Norway but also from the British Isles.

Anthropological studies on skulls from the time of settlement and blood group investigation of the present inhabitants show a

Table A III 1

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Number of men invited to participate (participants) in each age-group - and the percentage of participants investigated

Birth -year	Age 1968	Particip invited N	Particip investigated Response			Corrected response 1/ %
			N	%	%	
1907	61	128	87	68 0	68 0	69 6
1910	58	131	86	65 6	73 6	74 3
12	56	160	128	80 0		
1914	54	167	127	76 0	75 2	75 7
16	52	162	127	78 4		
17	51	172	125	72 7		
18	50	159	117	73 6		
1919	49	162	127	78 4	77 0	77 3
20	48	222	175	78 8		
21	47	225	173	76 9		
22	46	203	150	73 9		
1924	44	207	167	80 7	75 2	75 4
26	42	221	164	74 2		
28	40	225	160	71 1		
1931	37	212	149	70 3	70 3	71 3
1934	34	199	141	70 9	70 9	71 6
Total		2955	2204	74 6%		75 1%

1/ Calculated after exclusion of those who had emigrated or died from Dec 1 1966 to Nov 30 1967 according to the National Roster on Dec 1 1967

greater similarity to the Scottish and Irish people than to the Scandinavians [9] [10]

Iceland was a sovereign state but came under the Norwegian crown in 1262 and later under the Danish crown until it became a republic in 1944

Icelandic culture is essentially Scandinavian. However for centuries there have been commercial and cultural relations with West European countries especially Britain [11]

The area selected for this survey lies on the south west coast of Iceland and includes the capital Reykjavik, and five of the neighbouring communes. It lies within 11 kilometers from the Heart Preventive Clinic in Reykjavik where the investigation was performed

There has been a considerable and fairly constant migration to the Reykjavik area in the first half of this century from all parts of the country. In the year 1901 about 10% of the nation lived in the Reykjavik area compared to 60% in 1960 and 62% 1968

A computerized register The National Roster was established in 1953 based on the National Census of Dec 1st 1950 and a population register on Oct 16th 1955. The National Roster is recorded on Dec 1st each year

The total population of Iceland was on Dec 1st 1967 about 200 000. At that time about 105 000 were living in the Reykjavik area [12]

The selected population (participants) for the health survey comprised sixteen age groups born respectively in 1907
10 1 14 16 17 18 19 20 1 22 24 26 28
31 and 34 totalling approx 8500 persons [1]

One third of each group was invited to attend the study. Only those born on the 1st 4th 7th 8th and 31st day of each month. Altogether there were 2955 male participants in stage I aged from 34 to 61 years

Each age group was divided into three groups A B and C according to birthdays. Group B was invited to participate in stage I

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The total population of Iceland was on Dec 1st 1967 about 200 000. At that time about 104 000 were living in the Reykjavik area [12]

The sample population selected (participants) for the health survey comprised sixteen age-groups born respectively in 1907 10 12 14 16 17 18 19 20 21 22 24 26 28 31 and 34 totalling approx 8500 men [1]

From each group was invited to attend the survey equally those born on the 1st 4th 7th 28th and 31st day of each month^{1/}. Altogether there were 2955 male participants in the study aged from 34 to 61 years.

1/ The age groups were divided into three groups A B and C according to birthdays. Group B was invited to participate in the study.

Table A III 2

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Marital status^{1/} in each cohort of males (%) invited to participate
 (participants) in the first stage of the survey - and percentage
 of participants investigated (% response) in each subgroup

Cohort		Marital status				Total
Age		Unmarried	Married	Widowers	Divorced Separated	% N
61	% particip	21	70	5	4	100 12
	% response	37	79			
58 56	% particip	11	80	2	7	100 29
	% response	48	79			
54 52	% particip	18	76	2	5	101 66
	% response	53	82			
49 48	% particip	13	81	1	5	100 81
	% response	57	81			
44 42	% particip	14	81	0	5	100 65
	% response	46	82			
37	% particip	17	76	1	6	100 212
	% response	39	80			
34	% particip	17	78	1	5	101 199
	% response	46	77			
Participants N		443	2327	31	153	2955
Response %		49 2%	81 1%	62 5%	50 3%	74 6%

1/ According to the National Roster Dec 1 1966

Response

In early November 1967 invitations were mailed to all participants wherein they were asked to indicate whether they were prepared to attend a health survey in search for cardiovascular diseases sometime during the period from November 1967 to June 1968.

The letter of invitation stated that the survey was free and included an outline of what it involved.

The survey was publicized through newspapers and radio.

A second invitation identical to the first one was in March 1968 mailed to those who had not yet replied to the first one.

Altogether 96% of those who had accepted the first or the second invitations did attend the clinic. This corresponded to some 68% of all participants.

From August to November 1968 about two-thirds of those who had not replied to either invitation were contacted by telephone. In this way some 8% of the participants were persuaded to attend.

During May and June 1969 a few men were contacted by telephone and about 2% of the participants subsequently attended the clinic.

The clinic did not organize any transport for the participants to or from the clinic nor did it pay any expenses incurred in this respect.

The lowest response in individual age groups was 53% among the 51 year old males and the highest 81% among 44 year old

males. If the age groups are pooled into 5 year cohorts 30-34 years old 35-39 year old etc. the response rate is highest 77% among 45-49 year old males but tends to be lowest in the youngest and the oldest age groups. The overall response rate is 74.6% for 2203 participants. Discounting those participants who were recruited before December 1st 1967 the lowest response rate for one cohort is 70% and the overall response 75.1% but none of the men died had previously attended nor had any

of those who had emigrated Further information on attendance can be seen in Table A III 1 page 8

Non-response

The religion and marital status of all participants are known Some 83% of the males invited to participate in Stage I belonged to the National Church (Lutherian) according to the National Roster on Dec 1st 1966 some 10% belonged to Free Churches and some 6% belonged to no religious sect Attendance in individual age groups seems independent of religious affiliation

As regards marital status the situation is different Some 81% of married men attended the clinic whereas attendances among unmarried men and divorced men were similar or about 50% Further information on attendance with respect to marital status can be seen in Table A III 2

Collection of blood samples

From November 1967 to June 1968 about 20 men were systematically given an appointment to attend the clinic each weekday These were issued by letter some 10 days in advance to men who had accepted an invitation The appointments were arranged in such a way that as far as possible all age groups would be equivalent with respect to time of day weekday and time of year on which the appointments were given ^{1/} [1]

Participants were instructed not to eat or drink after 22⁰⁰ hrs on the night before their visit to the clinic If a participant had not fasted for the required period of time he was asked to come again later to give a blood sample

Blood samples were collected between 8³⁰ hrs and 10³⁰ hrs The participant lay down on a couch where an electrocardiogram was taken and blood pressure measured This took some 3 minutes After this the cuff of the sphygmomanometer was reinflated to compress the veins A nurse then collected blood from an arm vein into three Vacutainer B-D tubes (Beckton Dickinson & Co

1/ This refers to the first visit to the clinic but participants were expected to reattend the clinic some 10 days after the first visit

U S A) The tubes were sufficiently air-evacuated to draw 4-5 ml of blood into the first two tubes and about 15 ml into the third (The blood lipid analyses were done on blood from the last tube) Blood sampling took 1 2 minutes

Chemical analyses

Blood samples reached the laboratory before noon and soon after that separation of serum commenced For this the samples were centrifuged for 10 minutes at 2000 2200 r p m (1000 G) in a MSE super Medium centrifuge Occasionally further centrifugation was needed for ad quat separation On the other hand nothing was done about haemolysis Subsequently 2 7 ml serum were decanted into 10 15 ml test tubes When serum was poorly separated the transfer was done by means of a Pasteur pipette instead of decantation The tubes were then stored in a refrigerator at approximately 4 C with aluminium foil covering the row of tubes

Beta lipoprotein in serum were determined after precipitation with antisera Antihuman Beta Lipoprotein Precipitin Serum obtained from Hyland Laboratories Los Angeles The precipitation was carried out by mixing one drop of serum with two drops of antisera on a plastic coated card The mixture was subsequently drawn into glass capillaries which were then sealed in a gas flame The precipitate was spun down in the capillary in an Adams Autocrit Haematocrit centrifuge at 12600 r p m for 5 min (15 500 G) The depth of the precipitate was then measured on a millimetre scale with the aid of a magnifying glass to an accuracy of 0 1 mm

The equipment used for the determination with the exception of the centrifuge was obtained from Hyland Laboratories and the emptying directions were followed precisely (See Appendix page 144)

Triglycerides were determined fluorimetrically according to the procedure of Kessler and Lederer [13] This semiautomatic method involves saponification of triglycerides in an isopropanol

extract The subsequent oxidation of glycerol leads to the formation of formaldehyde which reacts with ammonia and acetylacetone to form a fluorescent compound 3 5 - diacetyl - 1 4 - dihydrolutidine The excitation wavelength was 405 nm and the fluorescence was measured at 480 nm

Extraction was carried out after noon on the day blood samples were centrifuged The time which elapsed from collection of the first blood sample until extraction had been completed could be up to 7 hours

A Folin pipette was used to transfer 0.5 ml of serum into 9.5 ml of isopropanol which had been dispensed by means of an Exelo automatic pipette The tube was gently agitated with a Vortex mixer closed with a screw cap containing a teflon sealing disc and then shaken vigorously for about 15 seconds, this length of time being estimated by counting to 15 Culture tubes 16 x 125 mm were used

The closed tubes were subsequently allowed to stand in a tube rack for at least 20 min (20-60 min) Some 2 g of zeolite mixture were then dispensed into each tube and the tube rack shaken vigorously several times by hand whereupon it was left with the tubes closed at room temperature overnight Every other day the tubes had to wait 24 hours longer and occasionally 2-3 days longer due to holidays

The tubes were centrifuged early in the morning at 2000 - 2200 r p m (1000 G) for 10 minutes

Reagents used for the triglyceride determination were as follows

Isopropanol (Isopropyl Alcohol Shell Chemicals See Appendix page 143) was obtained from the Government Pharmaceuticals Dept in drums containing 5 imp gallons

When needed some 4.5 litre of the isopropanol were shaken with 0.5-0.10 g of sodium borohydride (zur synthese kat no 6373 E Merck AG Darmstadt) to remove aldehydes and the mixture was subsequently distilled from a 5 litre flask at 82-83 °C in an all glass apparatus

The redistilled isopropanol was stored in a 4.5 litre flask

and lasted up to one month. No special test for aldehydes was carried out after distillation.

The zeolite mixture served to remove phospholipids and glucose which interfere with the triglyceride determinations.

The zeolite mixture was prepared by crushing 500-600 grams of zeolite (W. Z. Tylor Co. Baltimore Md. USA) in a Waring Blender followed by drying for 4 hrs at 110°C in an oven. To 100 parts (by weight) of the dried zeolite were added with thorough mixing 10 parts of Lloyd's Reagent (Hartman-Leddon Co. Philadelphia, USA), 10 parts calcium hydroxide ($\text{Ca}(\text{OH})_2$, pro analysi; E. Merck AG Darmstadt, Germany) and 2.7 parts of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, pro analysi; E. Merck AG Darmstadt, Germany).

Prior to each run the analyses of triglycerides on the AutoAnalyzer, three reagents were prepared.

The base reagent was prepared as follows: 6 g potassium hydroxide (P. 248 u.s.p. pellets; Fischer Scientific Co. and Eimer and Amend, USA) were weighed out on a top loading balance dissolved in 250 ml of deionized water and mixed with 750 ml of isopropanol.

The sodium metaperiodate reagent was prepared as follows: 2.13 g of sodium metaperiodate (NaIO_4 , MW 213, zur Bestimmung vic Hydroxylgruppen; E. Merck AG Darmstadt, Germany) were weighed out on a top loading balance and dissolved in 45 ml (in this measurement the last digit was estimated) of acetic acid, pro analysi (100%), E. Merck AG Darmstadt, Germany) and 354.6 ml of deionized water. The mixture was stirred with a glass rod until the constituent had dissolved.

The acetylacetone reagents were prepared as follows: 10 ml isopropanol and 3 ml acetylacetone ($\text{CH}_3\text{COCH}_2\text{COCH}_3$, reiner MW 100.12; E. Merck AG Darmstadt, Germany) were measured out by means of a graduated pipette and mixed by taking 400 ml of an ammonium acetate solution pH 5.8. 5.2 were then added. The ammonium acetate solution was prepared by first mixing 0.5 ml deionized water with 228 ml of acetic acid (as above) in a 2 litre brown screw cap bottle and then adding 290 ml of 26% ammonia.

TRIGLYCERIDES

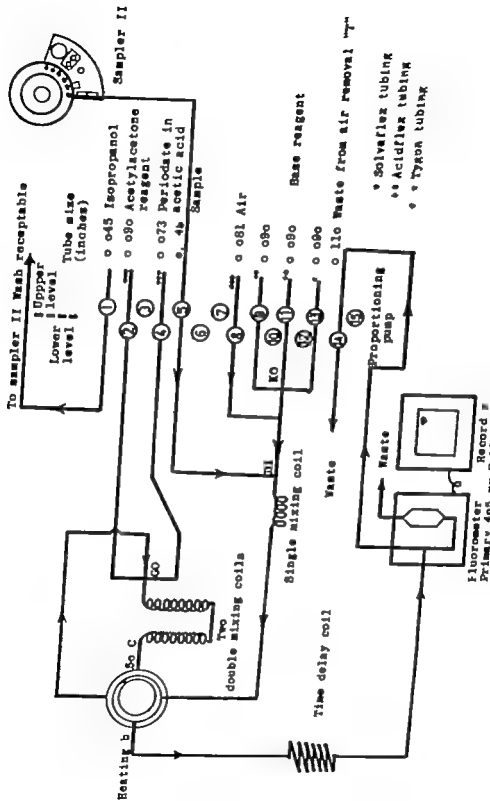


Figure A III 2 health survey in the Reykjavik area Stage I 1967 - 88 - Men
AutoAnalyzer flow diagram for the determination of serum triglycerides

(E Merck AG Darmstadt Germany) The mixture was allowed to cool and was then diluted to the 2 litre mark on the bottle with deionized water. The pH of this solution was checked before use. The ammonium acetate solution was sufficient for several runs.

Deionized water was prepared by passing tap water through an ion exchange resin bed in an Elgastat apparatus (Type C 203).

Standards were prepared from triolein redest (pure, Fluka AG Buchs Switzerland) which was obtained in 50 g lots and stored at 4°C.

Stock standard was prepared by first weighing out 1.000 g of triolein in a 100 ml beaker on an analytical balance and dissolving this in about 50 ml isopropanol. The solution was then transferred with 2-3 isopropanol rinses to a 100 ml volumetric flask and diluted to 100 ml.

Intermediate standards were prepared by weighing out respectively 3.75, 7.5, 15.0 and 22.5 g of the stock standard and diluting with isopropanol to a weight of 75 g. The scale on the balance was accurate to the nearest 0.1 g so the last digit had to be estimated. These standards were stored at room temperature in 100 ml brown screw cap bottles.

Working standard were prepared prior to every other run. For each of these standards 0.5 ml of the intermediate standards previously described were diluted with 8 ml of isopropanol and 0.5 ml of deionized water. The concentration of these standards corresponded respectively to 50, 100, 200 and 300 µg/l of triglyceride in serum.

Total cholesterol was determined colorimetrically according to the procedure described in the Technicon AutoAnalyzer Method 1 by H₂W (Appendix pag 186). This is a automated method was originally described by Block, Jerrett and Levine [19] and was based on an earlier method of Levine and Zak (1964) [20]. The basis of the method is the reaction of cholesterol with ferric chloride in a mixture of concentrated sulphuric acid and glacial acetic acid at 90°C resulting in the formation of a coloured compound with a maximum absorbance at 550 nm.

TRIGLYCERIDES

16

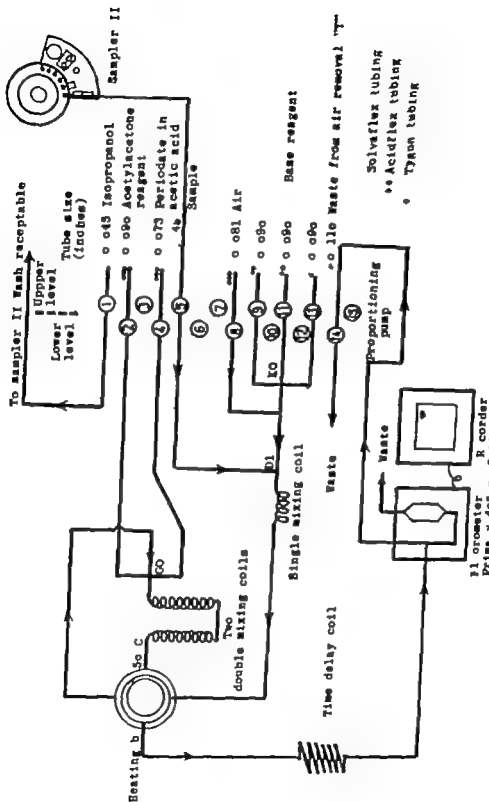


Figure A III 2 health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Autoanalyser flow diagram for the determination of serum triglycerides

Extraction (see page 14)

Reagents used for the cholesterol determination were as follows

Isopropanol (see page 14)

Ferric chloride reagent. This was prepared by weighing out on an analytical balance 1.375 g of ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ MW 270.30 E Merck AG Darmstadt Germany) and dissolving this in 2 litre acetic acid (pro analysi (100%) E Merck AG Darmstadt Germany) followed by addition of 1 litre of sulphuric acid (pro analysi (95-97%) E Merck AG Darmstadt Germany). This reagent was stored in 1 litre brown screw cap bottles.

Standards were prepared from pure cholesterol (cholesterin $\text{C}_{27}\text{H}_{46}\text{O}$; E Merck AG Darmstadt Germany)

A stock standard was prepared by dissolving 500 mg of cholesterol in about 50 ml of isopropanol in a 100 ml baker transferring the solution quantitatively with 2-3 isopropanol rinses to a 100 ml volumetric flask and making up to the mark with isopropanol. The stock standard was stored at room temperature in a brown 100 ml screw cap bottle.

Working standards were prepared by diluting respectively 1, 2, 3 and 4 ml of stock standard to 100 ml with isopropanol. The concentration of these standards corresponded to serum cholesterol concentrations of respectively 100, 200, 300 and 400 mg%. They were stored at room temperature in brown 100 ml screw cap bottles.

Equipment. A Tchnicon AutoAnalyzer I was used. This included the following main components: Sampler II, fixed speed proportioning pumps, adjustable heating baths, a colorimeter with a 15 mm tubular glass flow cell, a fluorimeter I and an 11 inch double pen recorder (Elliot Process Automation Ltd. England).

AutoAnalyzer Runs for cholesterol and triglyceride determination were carried out every other working day.

At the start of a run the cholesterol manifold was rinsed by first pumping through water for 5 minutes and then air for 3 minutes. Reagents and isopropanol were then pumped through for

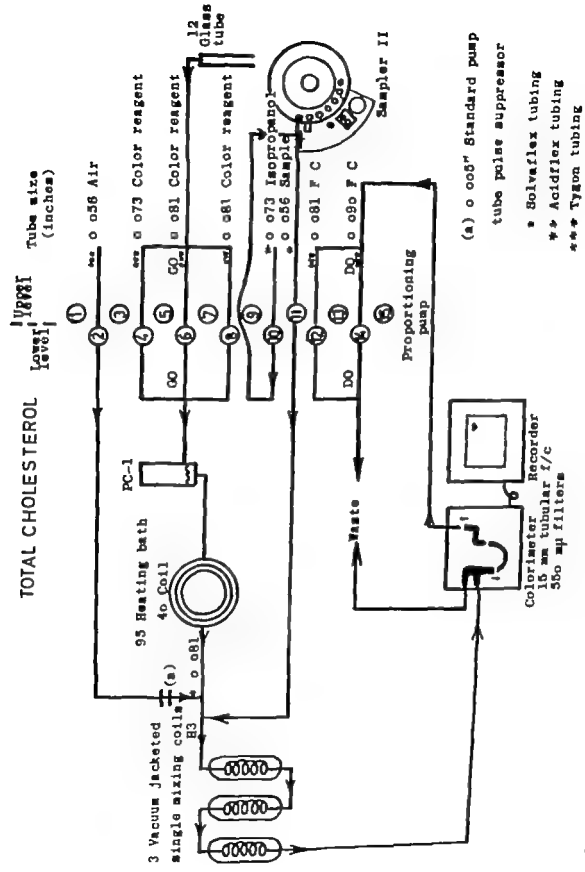


Figure A III 3 Health survey in the Reykjavik area Stage I 1967 - 68 - Men
total/zer flow d agram for the determination of serum total cholesterol

Triglycerides and total cholesterol Quality control was maintained on the cholesterol and triglyceride determinations. For this Control serum from Hyland Division, Travenol Laboratories Inc. California USA and Seronorm from Nyegaard & Co. Oslo Norway were used. Both were obtained from Bie & Berndsen Copenhagen Denmark in freeze dried form and imported by air. They were transported in non insulated packages but supplies were placed in a refrigerator at 4 C immediately upon arrival at the clinic.

From January 31st to May 27th 1968 Special Clinical Chemistry Control Serum was used to monitor the triglyceride and total cholesterol determinations.

From June 6th to July 4th Normal Clinical Chemistry Control Serum was used to monitor total cholesterol determinations.

From July 9th to September 24th Seronorm was used to monitor total cholesterol determinations.

Hyland serum was prepared for use by dissolving the contents of one ampoule in 5 ml of deionized water. The solution was then transferred to about 8 x 1.5 ml cups and stored in a deep freeze cabinet (-20 to -18 C). For each lot of extractions one cup was usually thawed and the control serum extracted at the same time as and in an identical fashion with the participants serum samples. During runs the participants serum extracts were interspersed with one and occasionally two or three control serum extractions.

The Nyegaard serum was treated in a similar fashion.

The results from the quality control program are shown in tables A III 3 & 4. Their chief statistical characteristics are shown in table A III 5 page 24. Fig A III 4 on page 8 is a scatter diagram of the results obtained from Feb 1st to May 27th.

Of 26 values for triglycerides in the Hyland Special Control Serum 8 (32%) fall outside the range (65-100 mg%) reported acceptable by the manufacturers. All of these exceeding the upper limit. The mean value was 62.9 mg% and the standard deviation was 7.43 mg% with 2 degrees of freedom. The mean value is thus

8 minutes 1 through the cholesterol system and 3 through the triglyceride system After this the baselines for cholesterol and triglycerides were adjusted to 99%T and 8-12% respectively

Class tubes of 2 ml capacity were used on the sampler tray These were placed in the plastic sample cup on the tray Because of their small size more sample had to be added during aspiration Due to evaporation only four samples were left on the tray at any one time Cholesterol and triglyceride standards in order of increasing concentration were included at the beginning and the end of each run Between every ten or so serum samples one of each series of standards (but never the highest one) were inserted The rate of sampling was 40 per hour and the sample to wash ratio was 2:1 The between-samples wash was isopropanol ^{1/}

The cholesterol manifold (see fig 3) Tygon pump tubing was used except for Solvaflex tubing for the sample A twelve inch long Acidflex tube connected the outlet from the water bath to an H3 fitting The tube was replaced when it started to deform or about once every two months

The triglyceride manifold (see fig 2) Tygon pump tubing was used except for Acidflex tubing for the base reagent and Solvaflex tubing for the sample isopropanol and waste In between the heating bath and the colorimeter was a 40 foot delay coil 1 1/2 mm in internal diameter

The recorder chart reading was taken on the same or the next day and the results recorded on the appropriate station cards A standard curve was drawn on a transparent plastic card through the standard peaks with the aid of a plastic coated lead ruler and this curve was subsequently used to determine the concentration in the samples If peaks exceeded the highest standard peak on the card the corresponding samples were measured again after dilution from 1:1 to 1:4 as needed

Quality control

Beta-lipoproteins No quality control of the beta lipoprotein measurements was carried out

1/ Before triglyceride determinations were started between sample washes were water otherwise isopropanol was used

Table A III *

Health survey in the Reykjavik area Stage I 1967 - 68 Men
 Values of total cholesterol in Hyland's Normal Clinical Chemistry
 Control Serum and in Nyegaard's Seronorm

Date of extraction	Hyland	Nyegaard
	Total cholesterol values in mg% ^{1/}	Total cholesterol values in mg% ^{2/}
8th June 1968	196	
11	189 192	
12	191	
19	188 190	
0	190	
21	190	
27	188	
28	184	
2nd July 1968	186	
4th	183	
9		90
10		94
12 Aug		94
15		100
16		81
23		96
27		100
29		83
2nd Sept		95
4th		86
5		100
11		93 95
13		97
17		96
20		99
24		96

1/ Accepted values 195 - 10 mg%
 2/ Recommended value 100 - 108 mg%

Table A III 3

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Values of total cholesterol and triglycerides in Hyland's
 Special Clinical Chemistry Control Serum

Date of extraction	Hyland	
	Total cholesterol values in mg% ^{1/}	Triglycerides values in mg% ^{2/}
31st Jan 1968		62
1 Feb	156	61 60
6th "	174	60
28 " "	174 176	
29 " "	166 160	70
14 Mar	174 174	75
18 "	166	60
19 "	167	59
20 "	162 170 165	62
21st "	158	59
22th "	162	60
29 " "	175	62
1st Apr	161	50
2nd		71
4th "	170	
8	166	69
17	172	72
18 "	172	
19	170	55
3d May	171 158	70 50
6 "	156	50
7 "	170	70
10	150	50
17 "	170	65
20	161	73
27	167	62
1/ Acceptable values	180 \pm 10 mg%	
2/ Acceptable values	55 - 10 mg%	

significantly greater than 55 mg% the difference being 7.9 mg% (This and subsequent values for standard deviations are based on only the first recorded value for each extract)

Of 30 values for total cholesterol in Hyland's Special Control Serum 16 (53%) fell outside the range (180- 10 mg%) reported as acceptable by the manufacturers all of these falling below the lower limit. The mean value was 166.3 mg% and the standard deviation was 8.71 mg% with 23 degrees of freedom. The mean value is thus significantly lower than 180 mg% the difference being 13.7 mg%.

Of 12 values for total cholesterol in Hyland's Normal Control Serum one (8.3 mg%) fell just below the acceptable value (195-10 mg%) reported by the manufacturers. The highest value was 196 mg%. The mean value was 189.5 mg% and the standard deviation was 3.41 mg% with 9 degrees of freedom. The mean value is thus significantly lower than 195 mg% the difference being 5.5 mg%.

Of 17 values for total cholesterol in Nyegaard's Seronorm 14 (82%) fell outside the range (100 - 106 mg%) recommended by the manufacturers all of them falling short of the lower limit. The mean value was 94.3 mg% and the standard deviation was 5.7 mg% with 15 degrees of freedom. The mean value is thus significantly lower than 103 mg% the difference being 8.7 mg%. On the other hand 4 (24%) of the results fell outside the limits 103 ± 10 mg%.

Statistical comparison (Bartlett's test) of the standard deviations in the total cholesterol determinations shows that there is no significant difference ($p > 0.05$) between the results obtained with the different control sera ($\chi^2(2) = 4.98$ compared with the 95% fractile which is 5.99). The estimated common standard deviation is 5.73 mg% with 47 degrees of freedom.

From January 31st to May 27th 1968 both the triglyceride and the total cholesterol content were measured in all samples of Hyland's Special Control Serum. The correlation coefficient

Table A III 5

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
Values of total cholesterol and triglycerides in control serum

		Total cholesterol	Triglycerides
Hyland s Special Clinical Chemistry Control Serum			
31st Jan 1968 - 27th May 1968			
Number	n	24 1/	23 /
Mean	\bar{x}	166 3 mg%	62 9 mg%
Variance	s^2	45 07	55 17
s d	s	6 71	7 43
Min		150	50
Max		175	75
Range		25	25

Hyland s Normal Clinical Chemistry Control Serum
6th June 1968 - 4th July 1968

Number	n	10 3/	
Mean	\bar{x}	189 5 mg%	
Variance	s^2	11 61	
s d	s	3 41	
Min		184	
Max		196	
Range		12	

Nyegaard s Seronorm
9th July 1968 - 24th Sept 1968

Number	n	16 4/	
Mean	\bar{x}	94 3 mg%	
Variance	s^2	26 76	
s d	s	5 17	
Min		81	
Max		100	
Range		19	

1/ Acceptable values 180 \pm 10 mg% 2/ 55 \pm 10 mg% 3/ 195 \pm 10 mg%
4/ Recommended value 100 106mg%

significantly greater than 5 mg% the difference being 7.9 mg% (This and subsequent values for standard deviations are based on only the first recorded value for each extract)

Of 30 values for total cholesterol in Hyland's Special Control Serum 16 (53%) fell outside the range (180 ± 10 mg%) reported as acceptable by the manufacturers all of these falling below the lower limit. The mean value was 166.3 mg% and the standard deviation was 8.71 mg% with 23 degrees of freedom. The mean value is thus significantly lower than 180 mg% the difference being 13.7 mg%.

Of 12 values for total cholesterol in Hyland's Normal Control Serum one (8.4 mg%) fell just below the acceptable value (195 ± 10 mg%) reported by the manufacturers. The high st value was 196 mg%. The mean value was 189.6 mg% and the standard deviation was 3.41 mg% with 9 degrees of freedom. The mean value is thus significantly lower than 195 mg% the difference being 5.4 mg%.

Of 17 values for total cholesterol in Nyegaard's Seronorm 14 (82%) fell outside the range ($100 - 106$ mg%) recommended by the manufacturers all of them falling short of the lower limit. The mean value was 94.3 mg% and the standard deviation was 5.7 mg% with 16 degrees of freedom. The mean value is thus significantly lower than 103 mg% the difference being 8.7 mg%. On the other hand 4 (24%) of the results fall outside the limits 103 ± 10 mg%.

Statistical comparison (Bartlett's test) of the standard deviations in the total cholesterol determinations show that there is no significant difference ($p > 0.05$) between the results obtained with the different control sera ($\chi^2 = 4.96$ compared with the 95% fractile which is 8.99). The estimated common standard deviation is 5.73 mg% with 47 degrees of freedom.

From January 31st to May 27th 1958 both the triglycerid and the total cholesterol content were measured in all samples of Hyland's Special Control Serum. The correlation coefficient

Health survey in the Reykjavik area 1967-68

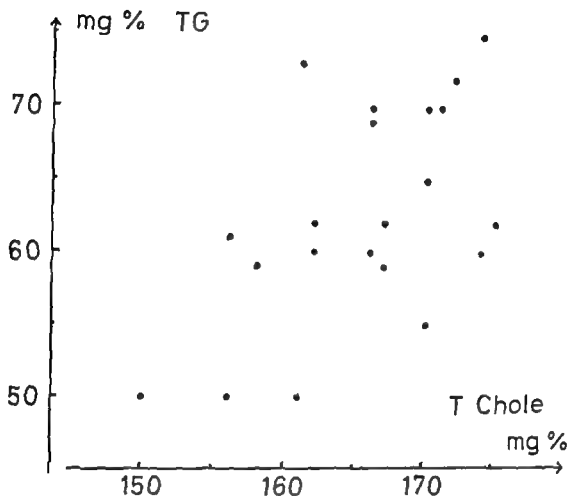


Figure A III 4 Scatter diagram showing values of total cholesterol and triglycerides in Hyland = Special Clinical Chemistry Control Serum Correlation coefficient 0.554

was 0.554 and the correlation therefore significant ($p = 0.01$ Fishers test) ^{1/}

Handling of data

All the results of the first visit were recorded on special forms termed station cards. The data were punched on cards as they became available for use in the computerized printout of medical reports and for data analysis. Punching was handled by IBM in Iceland but the printout of medical reports by the University Computer Centre (IBM 1620) and by IBM in Iceland (IBM 1401).

Before the participant returned for his second visit his medical report was available and had been checked by two physicians. During the medical examination a physician again checked through the participant's medical record and compared it with the station card and other basic documents as required.

All the data obtained in stage I of the mail survey are stored on magnetic tape. Data analysis with respect to this report was carried out at the Icelandic Computer Centre (IBM 360).

Before any analysis of data was started all the results were checked by the computer and any values which fell outside certain limits were printed out and compared with the original data for revision. The limits were as follows:

Beta lipoproteins	0.6 and 4.5 mmol/L
Triglycerides	12 310 mg/dl
Total cholesterol	140 - 600

¹ The serum results on total cholesterol were not begun until May. It should be mentioned that the standard deviation of 14 mmol/L for pooled serum from December 8th 1967 to January 19th 1968 was 7.87 mg/dl. See also the Appendix table A III 50.

RESULTS

In presentation of the results by tables and graphs the age groups have generally been collected in 5-year cohorts according to the accepted practice; thus 30 - 34 years 35 - 39 years etc.^{1/} Due to the way the age groups were selected the numbers of age groups in each cohort differ. Thus there is only one age group in the 30 - 34 cohort namely 34 year old men whereas in the 45 - 49 cohort there are four age groups. Reading of tables and graphs is thereby made easier but at the same time simplified. It should be noted that the number of values varies from one cohort to the next this being smallest in the youngest and the oldest cohorts. There are altogether 7 cohorts covering the age span from 30 to 64 years.

Physiological measurements rarely follow strictly a normal distribution but results from such measurements are however frequently normally distributed to an acceptable approximation. Where this is not the case their respective logarithms frequently are and the distribution is then said to be lognormal. In the present study it has been investigated whether the results conform more closely to a normal or a lognormal distribution.

In the preparation of tables and graphs there has been much standardization partly in order that a computer could be used efficiently.

A computer was used to prepare and print out the results in the following tables and graphs.

a) Frequency tables both single (Tables A III 6-9 and 12 age groups not collected in cohort) and double (Tables A III 15-35 age groups collected in cohort). In these tables the results were grouped. The tables are in a way basic to most other tables and graphs in this report.

b) Table of distribution functions (Tables A I I 7-10 and 13). The tables show what percentage of the results from each age group equal to or lower than (a) correct in stated values.

1/ A few sets of individual age group tables were prepared. These are presented in the Appendix. Such tables are not discussed in the main text but are occasionally referred to in footnotes.

Table A III 6 Health survey in the Refskjævik area Stage I 1967 - 68 - Men
Frequency table of serum B-lipoprotein values in men Total response 75 1^{1/}

Values in mm Immunocrit	Age in years														All age- groups		
	34	37	40	42	44	45	47	48	49	50	51	52	54	56		58	61
Class width 0.2 mm																	
- 9	1	0	0	0	0	3	1	1	0	0	0	0	0	0	0	0	3
1 0 - 1 1	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	1	1
1 2 - 1 3	0	0	0	0	0	3	2	3	1	0	0	2	0	1	0	0	5
1 4 - 1 5	1	2	1	0	0	3	5	1	4	1	4	1	4	1	2	0	17
1 6 - 1 7	1	3	3	3	3	2	6	4	4	5	5	5	4	4	2	0	37
1 8 - 1 9	4	4	4	7	9	3	13	13	4	7	5	10	9	9	3	6	81
2 0 - 2 1	6	3	4	13	10	6	13	13	4	7	5	10	9	9	3	6	121
2 2 - 2 3	4	8	11	9	10	12	3	6	3	3	3	1	1	6	2	7	89
2 4 - 2 5	3	7	6	6	4	5	3	3	2	4	6	3	3	3	1	1	65
2 6 - 2 7	2	1	3	1	3	4	2	2	1	0	1	0	2	2	1	0	25
2 8 - 2 9	4	1	4	4	3	1	4	4	3	2	1	5	2	0	3	0	41
3 0 - 3 1	1	3	2	0	5	3	2	9	0	3	4	1	1	0	0	0	34
3 2 - 3 3	1	1	0	2	1	0	3	1	0	1	0	0	0	1	0	0	13
3 4 - 3 5	1	1	1	1	0	0	0	1	0	0	1	0	0	0	0	0	4
3 6 - 3 7	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	5
3 8 - 3 9	0	1	0	0	0	0	1	2	0	0	0	0	0	0	2	0	6
4 0 - 4 1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2
4 2 - 4 3	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2
4 4 - 4 5	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	3
4 6	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	
Total (N)	33	38	48	47	51	60	46	52	23	27	29	27	33	27	16	19	556
N as % of no investigated	23.4	25.5	30.0	28.7	30.5	26.7	26.6	29.7	18.1	23.1	23.2	21.3	26.0	21.1	18.6	21.8	25.2

1/ % investigated Stage I started in Nov 1967 but the β -lipoprotein measurements began in May 1968. Because of the appointment system the 556 men can be considered as a stratified random sample of those investigated

Table A III 8

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Numerical characteristics of the distributions of serum B-lipo-
 protein values in men Total response 75 1^{1/}

B-lipoprotein values in mm Immunocrit	Age in years						
	34	37	40 42 44	46, 47 48, 49	50 51 52 54	56 58	61
Mean (\bar{x})	2 22	2 39	2 29	2 32	2 30	2 24	2 34
Variance	28	47	30	42	31	27	05
s.d. (s)	53	68	55	64	55	52	26
s/x	24	28	24	28	24	23	13
5% centile ^{2/}	1 48	1 44	1 63	1 46	1 63	1 56	1 34
10%	1 76	1 60	1 74	1 62	1 77	1 70	1 80
20%	1 84	1 83	1 84	1 85	1 89	1 89	1 83
30%	1 92	2 04	1 99	1 99	1 99	2 00	1 97
40%	2 02	2 21	2 10	2 28	2 06	2 07	2 04
50%	2 13	2 30	2 20	2 18	2 14	2 14	2 10
60%	2 29	2 40	2 30	2 31	2 37	2 25	2 14
70%	2 49	2 51	2 44	2 53	2 48	2 36	2 22
80%	2 77	2 98	2 72	2 85	2 77	2 58	2 27
90%	2 94	3 39	3 02	3 09	3 00	2 86	2 32
95%	3 22	3 77	3 26	3 35	3 13	3 32	2 36
Min	90	1 10	1 20	80	1 40	1 30	1 30
Max	3 40	4 20	4 50	5 00	5 50	3 80	2 40
Range	2 50	3 10	3 30	4 20	4 10	2 50	1 10
1 of ure (H)	33	38	146	161	116	43	19
1 % of no at g + d	23 4	25 5	29 7	25 8	23 4	20 1	21 8

1/ A nest started in Nov 1967 but the B-lipo
 protein measurements began in May 1968. Because of the appoint-
 ment of 536 men can be considered as a stratified random
 sample of those investigated.

Cut points below which are 5 and 10 20 etc per cent of
 the total

Table A III 7

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Cumulative distributions of serum B-lipoprotein values in men
 Total response 75 1%^{1/}

B-lipoprotein values in mm Immunocrit	Age in years						
	34	37	40 42 44	46 47 48 49	50 51 52 54	56 58	61
≤ 9	3 0	0	0	1 2	0	0	0
- 1 1	3 0	2 6	0	1 2	0	0	0
- 1 3	3 0	2 6	1 4	1 9	0	2 3	5 3
- 1 5	6 1	7 9	2 1	7 5	2 6	4 6	5 3
- 1 7	9 1	15 8	9 6	14 9	8 6	11 6	5 3
- 1 9	33 3	26 3	26 0	25 5	25 0	23 3	26 3
- 2 1	51 5	34 2	44 5	47 8	51 7	51 2	57 9
- 2 3	63 6	55 3	65 1	62 7	58 6	69 8	94 7
- 2 5	72 7	73 7	76 0	70 4	76 7	79 1	100 0
- 2 7	78 8	76 3	80 8	76 4	79 3	86 0	100 0
- 2 9	90 9	78 9	88 4	83 8	87 9	93 0	100 0
- 3 1	93 9	86 8	93 1	92 5	95 7	93 0	100 0
- 3 3	97 0	89 5	96 6	95 3	96 5	95 3	100 0
- 3 5	100 0	92 1	96 6	95 6	97 4	95 3	100 0
- 3 7	100 0	94 7	97 9	95 6	99 1	95 3	100 0
- 3 9	100 0	97 4	97 9	97 5	99 1	100 0	100 0
- 4 1	100 0	97 4	97 9	98 8	99 1	100 0	100 0
- 4 3	100 0	100 0	98 6	98 8	99 1	100 0	100 0
- 4 5	100 0	100 0	100 0	98 8	99 1	100 0	100 0
No of measurment (N)	33	38	146	161	116	43	19
N as % of no investigated	23 4	25 5	29 7	25 8	23 4	20 1	21 8

1/ % investigated Stage I started in Nov 1967 but the B-lipoprotein measurements began in May 1968 Because of the appointment system the 556 men can be considered as a stratified random sample of those investigated

Tabl A III B

Health survey in the Reykjavik area Stage I 1967 - 68 Men
 Numerical characteristics of the distributions of serum B-lipo
 prot in values in men Total response 75 1%^{1/}

B-lipoprotein values in mm Immunocrit	Age in years						
	34	37	40 42 44	46 47 48, 49	50 51 52 54	56 58	61
Mean (X)	2 22	2 39	2 29	2 32	2 30	2 24	2 34
Variance	28	47	30	42	31	27	06
s.d. (s)	53	68	55	64	55	52	26
s/x	24	28	24	28	24	23	13
5% centile ^{2/}	1 48	1 44	1 63	1 66	1 63	1 56	1 34
10%	1 76	1 60	1 74	1 82	1 77	1 70	1 80
20%	1 84	1 83	1 88	1 85	1 89	1 89	1 89
30%	1 92	2 04	1 99	1 99	1 99	2 00	1 97
40%	2 02	2 21	2 10	2 38	2 06	2 07	2 04
50%	2 13	2 30	2 20	2 18	2 14	2 14	2 10
60%	2 29	2 40	2 30	2 31	2 37	2 25	2 16
70%	2 49	2 51	2 44	2 53	2 48	2 36	2 22
80%	2 77	2 98	2 72	2 85	2 77	2 58	2 27
90%	2 94	3 39	3 02	3 09	3 00	2 86	2 32
95%	3 22	3 77	3 26	3 35	3 13	3 32	2 34
Min	90	1 10	1 70	80	1 40	1 30	1 30
Ma	3 40	4 20	4 90	5 00	5 30	3 80	2 40
Rang	2 50	3 10	3 30	4 20	4 10	2 50	1 10
No of me s rem (N)	33	38	144	161	116	43	19
Ma % of no n tigt r d	23 4	25 5	29 7	25 8	23 4	20 1	21 8

1/ % in st gated Stag I tarted in Nov 1967 b t the p lipo
 prote n mea urements began in May 1968 Becaus of the appoint
 ment yst n the 58 -en can be con id red as a tratified random
 sample of thos inv atigated

2 Cutting point b low which are found 5 10 20 etc per c nt of
 h i

Table A III 7

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Cumulative distributions of serum B-lipoprotein values in men
 Total response 75 %^{1/}

B-lipoprotein values in mm Immunocrit	Age in years						
	34	37	40 42 44	46 47 48 49	50 51 52 54	56 58	61
4 9	3 0	0	0	1 2	0	0	0
- 1 1	3 0	2 6	0	1 2	0	0	0
- 1 3	3 0	2 6	1 4	1 9	0	2 3	5 3
- 1 5	6 1	7 9	2 1	7 5	2 6	4 6	5 3
- 1 7	9 1	15 8	9 6	14 9	8 6	11 6	5 3
- 1 9	33 3	26 3	26 0	25 5	25 0	23 3	26 3
- 2 1	51 5	34 2	44 5	47 8	51 7	51 2	57 9
- 2 3	63 6	55 3	65 1	62 7	58 6	69 8	94 7
- 2 5	72 7	73 7	76 0	70 8	76 7	79 1	100 0
- 2 7	78 8	76 3	80 8	76 4	79 3	86 0	100 0
- 2 9	90 9	78 9	88 4	83 8	87 9	93 0	100 0
- 3 1	93 9	86 8	93 1	92 5	95 7	93 0	100 0
- 3 3	97 0	89 5	96 6	95 3	96 5	95 3	100 0
- 3 5	100 0	92 1	96 6	95 6	97 4	95 3	100 0
- 3 7	100 0	94 7	97 9	95 6	99 1	95 3	100 0
- 3 9	100 0	97 4	97 9	97 5	99 1	100 0	100 0
- 4 1	100 0	97 4	97 9	98 8	99 1	100 0	100 0
- 4 3	100 0	100 0	98 6	98 8	99 1	100 0	100 0
- 4 5	100 0	100 0	100 0	98 8	99 1	100 0	100 0
No of measuram (N)	33	38	146	161	116	43	19
N as % of no investigated	23 4	26 6	29 7	25 8	23 4	20 1	21 8

1/ % investigated Stage I started in Nov 1967 but the B lipoprotein measurements began in May 1968. Because of the appointment system the 556 men can be considered as a stratified random sample of those investigated.

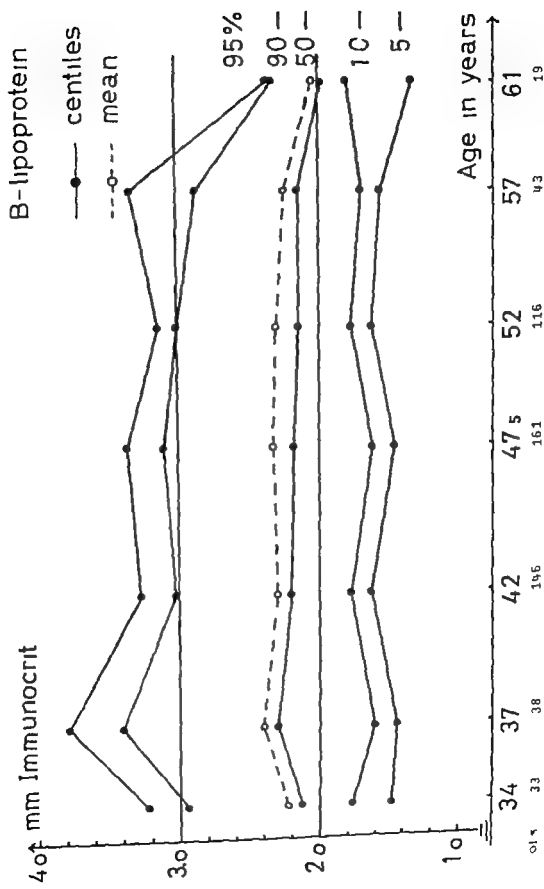


FIGURE A III 5 Health survey in the Reykjavik area Stage I 1967-68 - Men
Diagram showing the mean, median and a few centiles of the distributions of β -lipoprotein values
at 7 hr (34 yr) (37 yr) (40 yr) (42 yr) (46 yr) (47 yr) (48 yr) (49 yr) (50 yr) (51 yr) (52 yr) (54 yr)

The stated values are the right hand side end points of the classes in the frequency tables

c) Tables of chi square statistical characteristics of the results in each cohort. Mean variance, standard deviation, coefficient of variation, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 95% centiles, minimum and maximum value and the range (Tables A III 6, 11 and 14)

d) Histograms of the results from each cohort (Figs A III 6, 11 and 15). For preparation of histograms the computer grouped the result into 17 intervals and printed out the symbol \times for each percentage falling within each interval. The height of the column is therefore accurate to within $\pm 5\%$. The end columns represent results falling outside certain limits, these being the same for all 7 cohorts. Columns in these end intervals are separated from other columns by the symbol \sim ^{1/}. The histograms indicate the position and the shape of the density functions of the distribution functions of the result and how they change with age.

e) Fractile diagrams (Fig. A III 7, 12 and 16) of the distribution functions for the cohorts 30, 34, 40, 44, 50, 59 and 60, 64 years. In a coordinate system the ordinate scale is transformed in such a way that the graph of a normal distribution function is a straight line. In these fractile diagrams presented here the graph are not drawn but a few points from the graph of the (cumulative) distribution function of the results are plotted on the coordinate system. These points should lie near a straight line if the results are normally distributed. The points selected correspond to the right hand side end points of the 17 columns on the baseline of the aforementioned histograms. A point for the points where the values of the distribution function are 0.05 and 0.95. Similar fractile diagrams were also prepared for the logarithm of the result (Figs A III 8, 13 and 17)^{1/}

In addition to the above mentioned tables and figures this report contains three types of graphs

^{1/} See a more detailed description in the Appendix

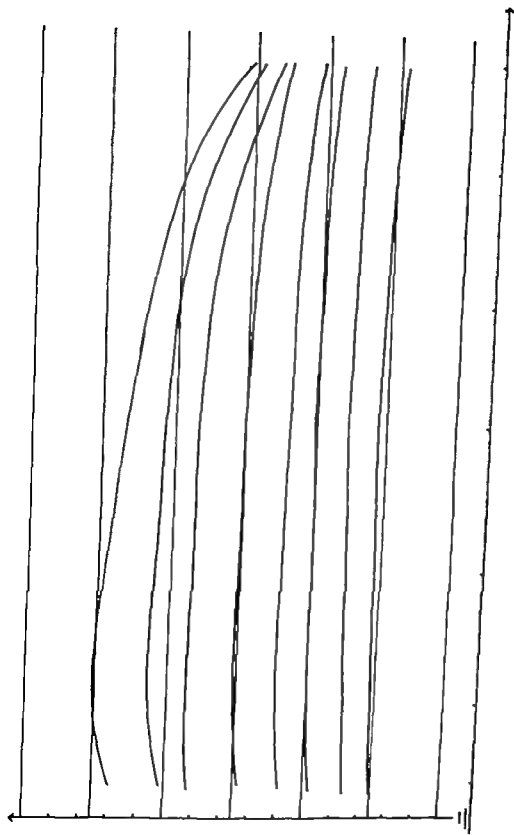
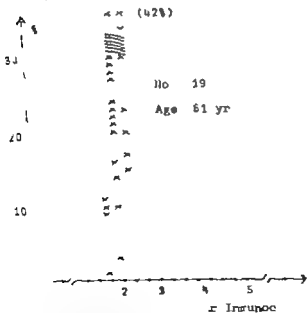
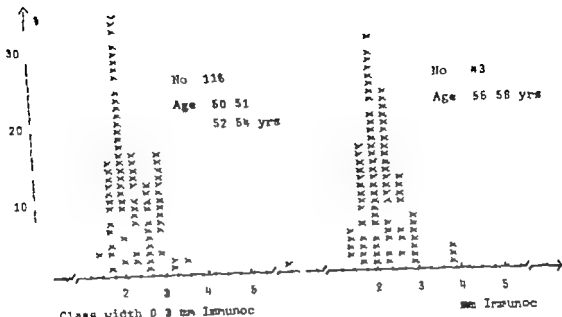


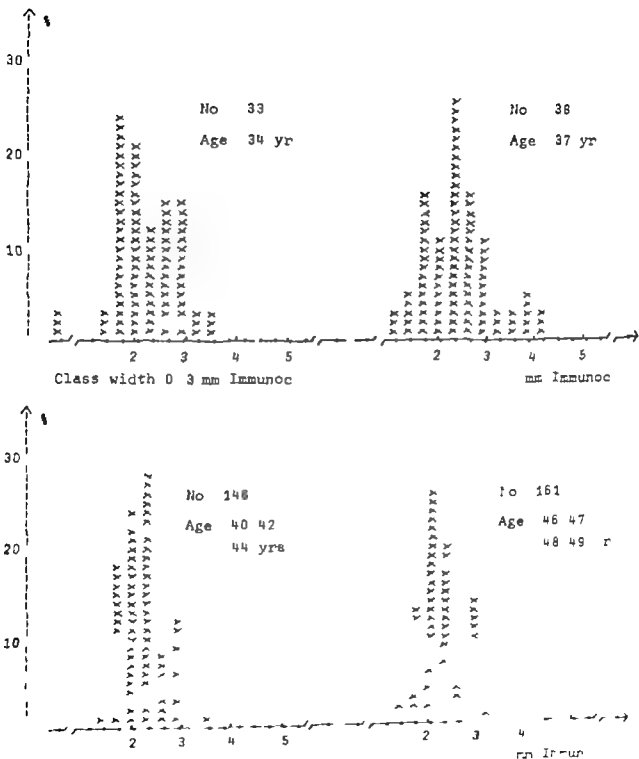
Figure A III 5b Lye fitted curves (Obat highly subjective) 5 10 30 50 70 80 90 95 100

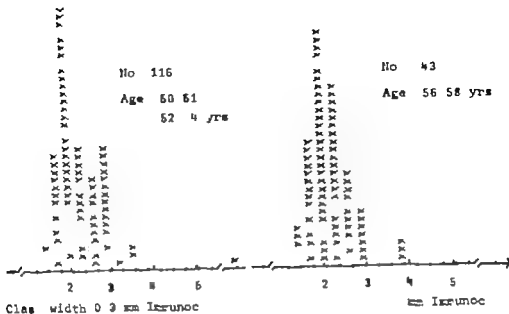


- 1/ The investigated Stage I started in Nov 1957 but the lipoprotein in sea remnants began in May 1958. Because of the appropriate temperature 56°C can be considered as a stratified random sample of those investigated.

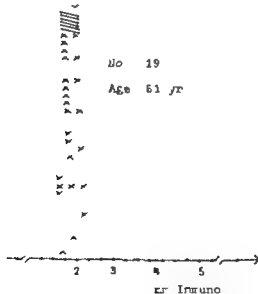
Figure A III 6

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
Histograms showing distributions of serum β -lipoprotein values in men
Total response 751 ^{1/}



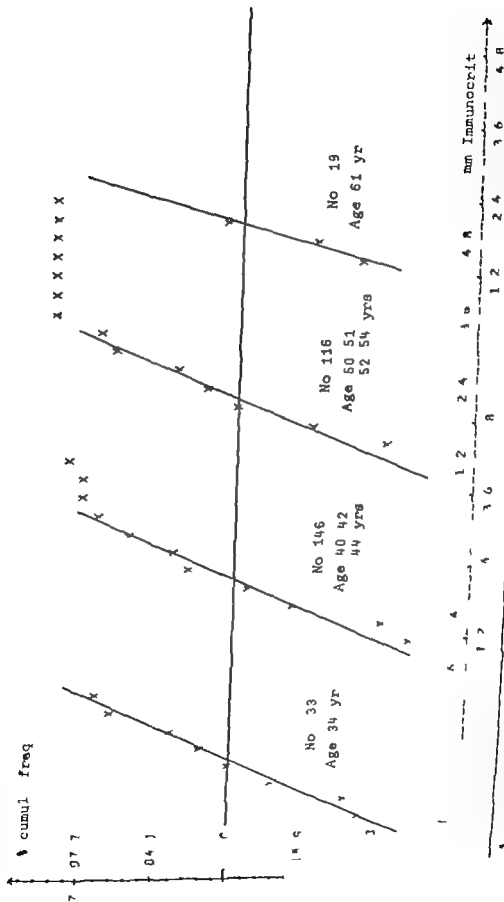


< x (42%)



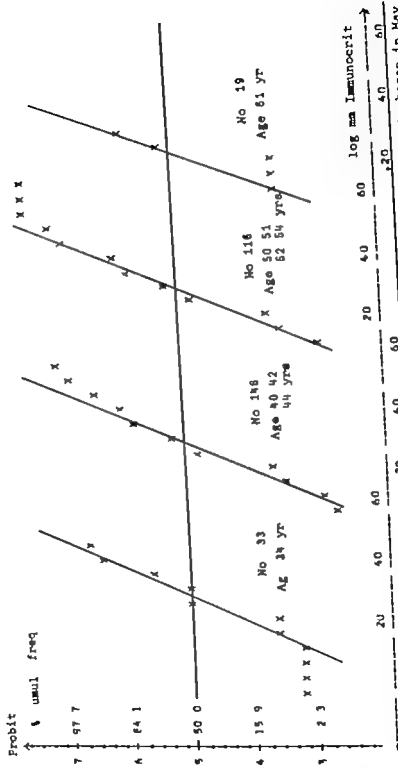
/ % in stighted t g l start d in No 1967 b t th A lipo
prot in mass rer t beg n i May 1968 Be ause of the
ppoint nt y t the 556 = be consid rd a tr ti
fi d na z arpl f thos in t g t d

Figure A III 7 Health survey in the Reykjavik area Stage I 1967-68 -Men
Fractile diagrams of the distributions of serum B-lipoprotein values in men Total number (No) 314
investigated in all four cohorts Response 478 1/



Investigated stage I started in Nov 1967 but the A-lipoprotein measurements began in May 1968 because of the appointment system the 314 men can be considered as a stratified random sample of those investigated

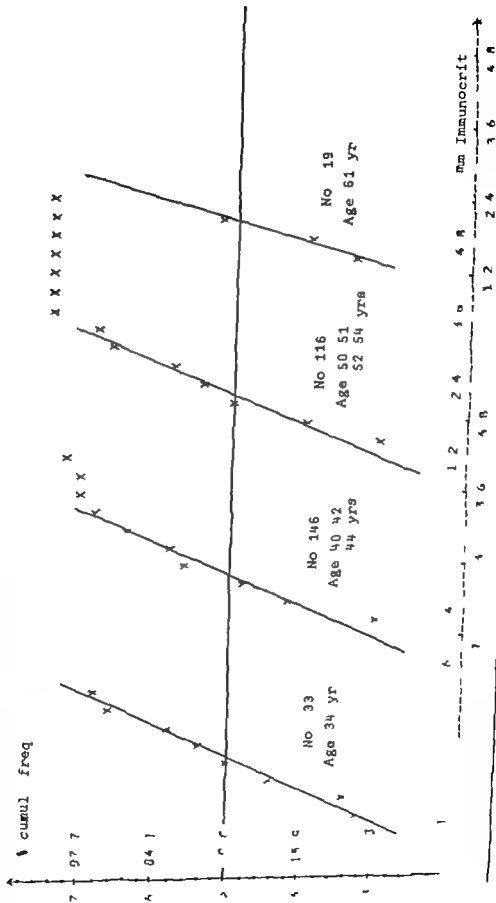
Figure A III a b lth rey in th Reykjavik area Stage I 1967 88 Men Total number
 Fra til di grams of th distributions of th logarithm of lipoprotein value s in men
 (N) investigated in 11 for horts 314 Response 74 78 1/



1/ Investigated Stage I started in Nov 1967 but the lipoprotein measurements began in May 1968. Because of the appointment system the 314 men can be considered as a stratified random sample of those investigated

Figure A III 7 Health survey in the Reykjavik area Stage I 1967-68 -Men

Fractile diagrams of the distributions of serum B-lipoprotein values in men Total number (No) 314 investigated in all four cohorts Response 74 7%



1/ investigated Stage I started in Nov 1967 but the A-lipoprotein measurements began in May 1968. Because of the appointment system the 314 men can be considered as a stratified random sample of those investigated.

f) Graphs showing the variation of the means and a few main fractiles with age (Figs A III 5 10 and 18) These were prepared by plotting on graph paper the values for each cohort according to the tables of chief statistical characteristics (see paragraph c)

g) Graphs showing the weekly means both standardized and not standardized together with the weekly standard deviations during the period from December 1967 to the beginning of July 1968 (Figs A III 9 10 and 19)

h) Graphs showing how the correlation coefficients vary with age (Fig A III 20) They are based on tables A III 15 - 35

Beta lipoproteins

Analyses were started by the middle of May Beta lipoproteins were measured in sera from 556 participants and these turned out to be between 20% and 30% of each cohort (Table A III 8 page 31) The results are presented as mm Immunocrit (abbreviated mm IC) to an accuracy of 0.1 mm

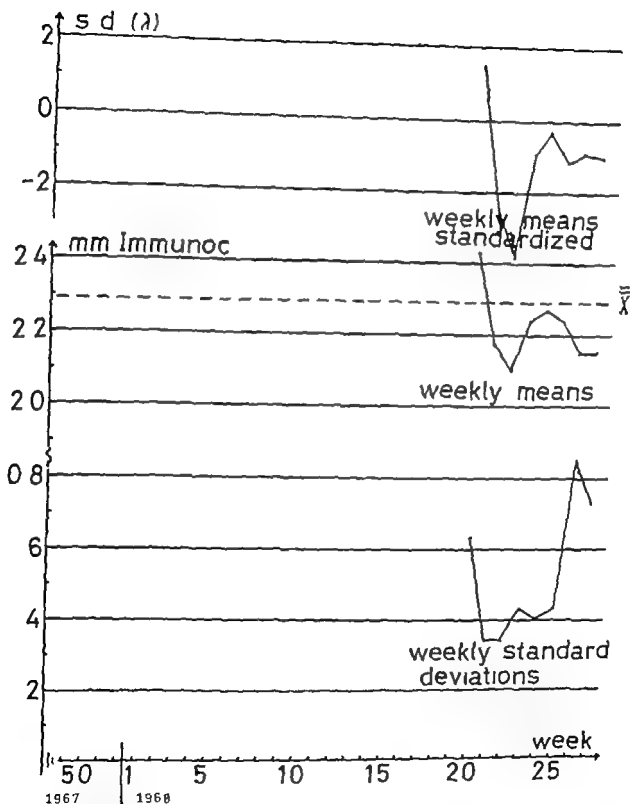
From fractile diagrams and the histograms (Figs A III 6 7) the results from each cohort appear to be approximately normally distributed There are however some deviations as seen clearly in fig A III 5 and as suggested by the histograms The distributions are slightly skew with a slightly longer tail towards the right except in the old age group This is shown by the fact that mean values are about 0.1 mm IC higher than the median value ^{1/}

The coefficient of variation varies between 0.23 and 0.28 except in the oldest cohort where it is 0.13 The logarithm of the results is therefore also approximately normally distributed as shown by the fractile diagrams (Fig A III 8) In fact the approximation appears to be closer to a lognormal distribution than to a normal distribution

Means 50% fractiles (medians) and lower fractiles appear to be a reasonable approximation to be age independent Thus the means 50% 10% and 5% fractile are about 2.3 2.2 1.7 and

1/ See also b_2 (coefficient of excess) in Table A III 45 in Appendix

Health survey in the Reykjavik area 1967 '68



Obs per week 26-65

Figure A III 9 Health survey in the Reykjavik area Stage I 1967 '68 - Men Weekly mean values (\bar{x}) weekly standard deviations (s) and weekly mean values standardized ($\lambda = (\bar{x} - \bar{\bar{x}}) \sqrt{n}/s$) of serum A-lipo protein values in men $n = \text{obs pr week}$ --- $\bar{\bar{x}}$ grand mean

1.6 mm IC respectively

The high r fractiles seem to be to a reasonable approximation age independent up to the age of 50 but to decrease considerably over the next decade this decrease being about 0.5 mm IC for the 90% and 95% fractiles (Fig A III 5 p 32). Thus the 90% and 95% fractiles are about 3.8 mm IC and 3.3 mm IC for the age interval 35-50 years.

Fig A III 9 p 38 shows graphs of the means (\bar{x}) and standard deviations (s) for each week in the period from the start of the survey in the beginning of December 1957 up to the summer vacation in the beginning of July 1958 where the number of weekly measurements was greater than 9. There is also a graph of the standardized weekly deviations (λ) from the grand mean (\bar{x}). These deviations are computer calculated according to the formula $\lambda = (\bar{x} - \bar{x}) / s$ where s is the weekly standard deviation and n is the number of results per week. As previously mentioned and as seen on the diagram beta lipoprotein determinations were not started until the middle of May 1958. 37% results or some 68% of the total number of results is from the period up to the summer vacation. (See a further note in the discussion).

Total cholesterol

Analyses were carried out from the beginning of the survey and continued throughout the period of the survey. Total cholesterol was measured in 2194 participants or 91.6% of those who attended the clinic. There are no results for 19 participants the reason being that a blood sample could not be collected in the time available or these samples were lost by accidental pilage. The results are presented as mg% (mg/100 ml) to an accuracy of 1 mg%.

From the fractile diagrams and the histograms the results appear to be normally distributed (Figs A III 11 12). There are however slight deviations as seen clearly in Fig A III 10 and 9. Evidenced by the histograms for the younger cohorts. The distribution are lightly skewed with a somewhat longer tail to the right. This is shown clearly by the fact that mean values are about 2-4 mg% higher than the median values.

Table A III 3 Health survey in the Reykjavik area Stage I 1967 - 68 - Men
Frequency table of serum total cholesterol values in men Total response 75 141

Values in mg/100 ml (mg%)	Age in years													All age- groups			
	34	37	40	42	44	46	47	48	49	50	51	52	54		56	58	61
Class width 10 mg%																	
161 - 170	2	1	0	2	0	1	0	0	0	0	2	3	0	1	1	0	13
171 - 180	3	5	3	3	1	2	3	3	1	2	1	2	0	1	1	1	16
181 - 190	7	8	6	4	2	4	2	3	0	2	1	2	0	1	1	3	36
191 - 200	15	7	8	11	6	3	7	5	6	2	2	5	4	3	5	3	72
201 - 210	6	8	9	2	15	8	17	5	7	3	3	4	5	4	1	3	95
211 - 220	10	12	15	10	20	11	19	13	11	8	7	8	7	12	2	3	107
221 - 230	10	12	15	19	16	11	13	13	11	11	4	7	10	9	2	5	154
231 - 240	18	19	17	16	10	11	16	22	16	8	14	9	8	7	9	6	183
241 - 250	14	22	19	18	16	11	16	20	10	14	17	12	7	5	7	14	202
251 - 260	14	13	12	14	13	23	10	15	10	14	17	16	19	20	12	6	207
261 - 270	6	13	12	14	13	9	15	12	11	11	13	12	7	10	10	3	236
271 - 280	11	7	10	10	15	10	13	16	15	7	11	8	14	11	4	3	171
281 - 290	7	10	8	9	10	14	12	14	15	12	6	5	15	9	5	4	167
291 - 300	3	7	10	8	8	5	8	9	5	8	12	7	5	2	7	10	151
301 - 310	2	3	3	7	3	6	4	9	4	4	3	7	9	7	0	3	111
311 - 320	4	3	4	3	2	11	5	3	3	1	2	5	7	3	4	4	71
321 - 330	2	2	1	7	1	0	3	2	2	1	1	5	4	2	1	1	50
331 - 340	1	0	2	1	3	2	2	1	2	2	7	1	4	2	3	1	41
341 - 350	2	0	1	1	3	5	2	4	0	3	0	0	2	2	4	1	34
351 - 360	1	0	0	2	0	0	0	1	0	0	1	0	0	1	1	1	24
361 - 370	0	1	2	0	0	2	0	0	0	1	0	0	0	1	0	1	9
371 - 380	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	8
381 - 390	0	1	0	0	0	2	0	0	0	1	0	0	0	1	0	0	4
391 - 400	0	0	1	0	0	1	2	0	0	0	0	0	0	0	0	0	6
401 - 410	0	1	0	1	1	1	2	2	2	1	0	0	0	1	0	0	6
Total (N)	141	149	160	163	165	149	172	175	127	117	125	126	127	126	85	87	2194
as % of no investigated	100	100	100	99	98	99	99	100	100	100	100	99	100	98	98	100	99

1/ 1 investigated

1/ 1 investigated

Tabl A III, 11

Health survey in the Reykjavik area Stage I 1967 68 - Men
 Numerical characteristics of the distributions of serum total
 cholesterol values in men Total response 75 1^{1/}

Total chole values in mg/100ml	Age in years						
	34	37	40 42 44	46 47 48 49	50 51 52 54	56 58	61
Mean (X)	241 99	246 14	249 84	257 74	257 01	256 28	255 18
Variance	1866 13	1713 40	1635 18	2034 69	1628 28	1934 08	2058 52
s d (s)	43 19	41 39	42 83	45 10	42 75	43 97	45 37
s/X	18	17	17	17	17	17	18
5 % centile	180 57	182 31	185 75	193 43	187 21	186 19	181 67
10 %	190 57	191 79	196 10	205 03	202 64	202 00	198 17
20 %	199 97	211 17	214 63	219 76	220 79	220 61	219 30
30 %	217 80	223 58	225 20	232 41	234 47	233 25	232 00
40 %	231 28	233 97	235 20	243 07	246 69	245 67	238 21
50 %	239 11	244 07	245 92	253 17	255 58	254 15	246 61
60 %	248 79	255 23	256 99	264 71	264 45	262 80	259 17
70 %	258 86	263 04	268 60	277 00	276 38	274 06	281 40
80 %	275 77	277 93	282 87	288 39	291 84	288 21	290 10
90 %	296 83	294 93	302 19	310 82	310 24	315 25	313 75
95 %	320 38	312 33	324 33	333 35	330 71	336 25	337 80
Min	144 00	156 00	158 00	158 00	136 00	156 00	168 00
Max	178 00	416 00	460 00	512 00	420 00	401 00	380 00
Range	234 00	260 00	302 00	354 00	284 00	245 00	212 00
No of me urem (N)	141	149	488	623	493	211	87
N as % of no inv stigat d	100 0	100 0	99 4	99 7	99 8	98 6	100 0

1/ % inv stigat ed

2/ Curti g points below which are found 5 10 20 etc per cent of
 the values

Table A III 10

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Cumulative distributions of serum total cholesterol values in men
 Total response 75 1^{1/}

Total cholesterol values in mg/100ml (mg%)	Age in years						
	34	37	40,42 44	46,47 48 49	50,51 52 54	56,58	61
≤ 160	1 4	7	4	2	1 0	9	0
- 170	2 8	7	1 2	1 0	1 4	1 9	1 1
- 180	5 0	4 0	3 3	2 2	2 4	2 8	4 6
- 190	9 9	9 4	6 6	4 0	6 3	6 6	8 0
- 200	20 6	14 1	12 7	7 4	9 1	9 0	11 5
- 210	24 8	19 5	16 6	13 2	13 3	15 6	14 9
- 220	31 9	27 5	24 8	20 5	19 8	19 9	20 7
- 230	39 0	35 6	35 9	28 2	26 9	28 4	27 6
- 240	51 8	48 3	44 7	37 4	34 7	34 1	43 7
- 250	61 7	53 0	54 5	47 5	43 2	45 5	54 0
- 260	71 6	67 8	63 5	56 8	56 6	57 8	60 9
- 270	75 9	76 5	71 5	64 4	65 2	67 3	64 4
- 280	83 7	81 2	78 7	73 0	73 3	74 9	69 0
- 290	88 6	87 9	84 2	81 9	79 0	81 5	80 5
- 300	90 8	92 6	89 5	86 2	86 3	85 8	83 9
- 310	92 2	94 6	92 2	89 9	90 1	89 1	88 5
- 320	95 0	96 6	94 1	93 4	92 5	91 0	93 1
- 330	96 5	98 0	96 5	94 5	94 9	93 4	94 2
- 340	97 2	98 0	97 3	96 1	97 4	96 2	95 4
- 350	98 6	98 0	98 4	97 4	98 6	97 2	96 5
- 360	99 3	98 0	98 8	97 6	98 8	98 6	97 7
- 370	99 3	98 7	99 2	97 9	99 0	99 0	98 8
- 380	100 0	98 7	99 2	97 9	99 4	99 0	100 0
- 390	100 0	99 3	99 4	98 2	99 6	99 5	100 0
≤ 400	100 0	99 3	99 6	99 0	99 6	99 5	100 0
No of measuram (N)	141	149	488	623	495	211	87
N as % of no investigated	100 0	100 0	99 4	99 7	99 8	98 6	100 0

1/ % investigated

Health survey in the Reykjavik area Stage I 1967-'68 - Men

mg% T Chole

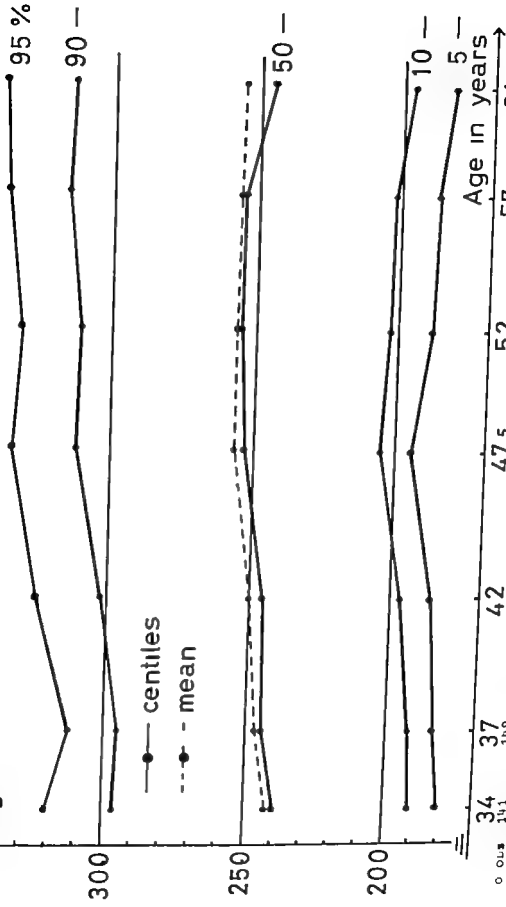


Figure A III 10 health survey in the Reykjavik area Stage I 1967 - 68 - Men
 diagram showing the mean median and a few centiles of the distributions of total cholesterol
 values in 7 cohorts (34 yr) (40 42 44 yrs) (45 47 48 49 yrs) (50 51 52 54 yrs)
 (56 58 yrs) and (61 yr)

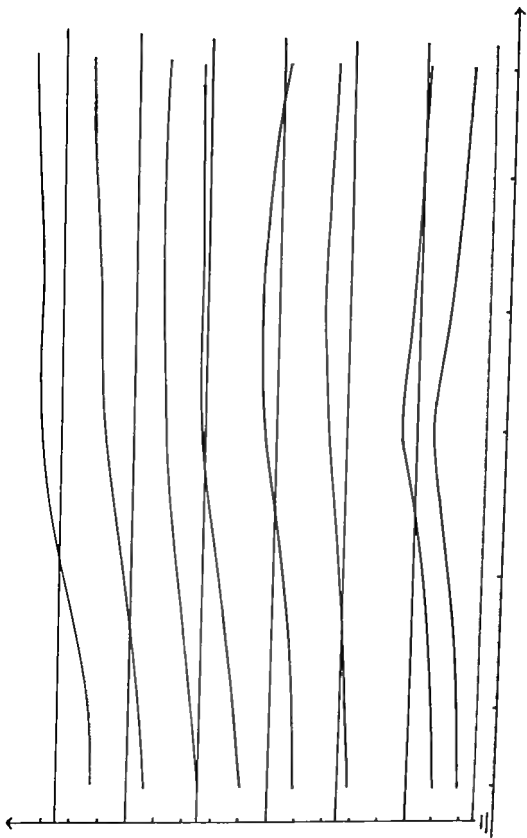


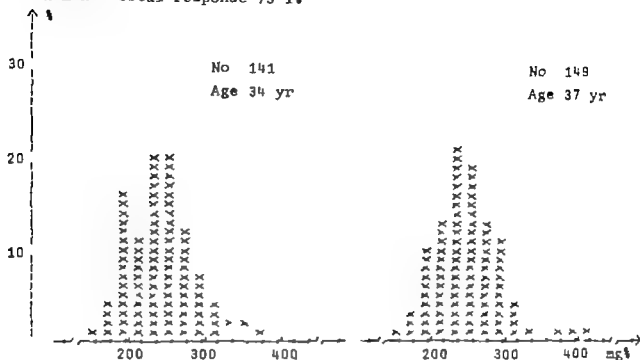
Figure A 111 10b Lys fitted curves (Obs highly subjective 10 30 50 70 80 90 95% centiles

The coefficients of variation are 0.17 except for the youngest and the oldest cohorts where they are 0.18. The logarithm of the results is therefore also approximately normally distributed as shown by the fractile diagrams (Fig. A III 13). In fact the approximation appears to be closer to a lognormal distribution than to a normal distribution.

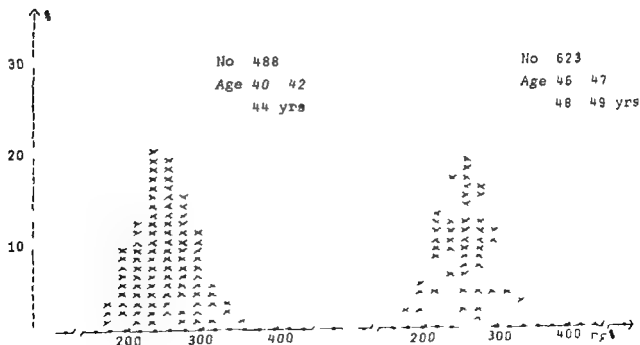
The mean values and all the fractiles appear to increase with age during the interval studied up to the age of 50. The increase is about 10-15 mg% or somewhat less than 1 mg% per year. Thus in the 34 to 49 year age interval the 95% fractiles increase from 315 mg% to 330 mg%, the 90% fractiles from 295 mg% to 310 mg%, means and medians from 240 mg% to 255 mg%, the 10% fractiles from about 190 mg% to 205 mg% and the 5% fractiles from about 180 mg% to 190 mg%. About the age of 50 there seems to be a change and the means and the fractiles are fairly steady during the next decade except that the lower fractiles appear to decrease by somewhat less than 1 mg% per year. Thus in the age interval 50 to 61 years the 95% fractile is about 330-335 mg%, the 90% fractiles are about 310-315 mg%, the mean and medians are about 255 mg%. However the 10% fractiles decrease from 205 mg% to 195 mg% and the 5% fractiles from 190 mg% to 180 mg% during the fifties.

Figure A III 11

Health survey in the Reykjavik area Stage I, 1967 - 68 - Men
 Histograms showing distributions of serum total cholesterol values
 in men Total response 75 1%



Class width 20 mg%



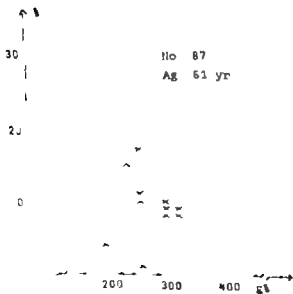
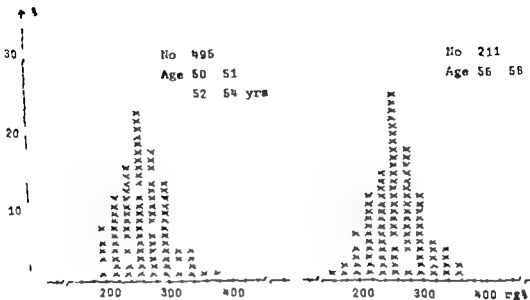


Figure A III 12 health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Fractile diagrams of the distributions of serum total cholesterol values in men Total number
 (No) investigated in all four cohorts 1211 Response 74 7%

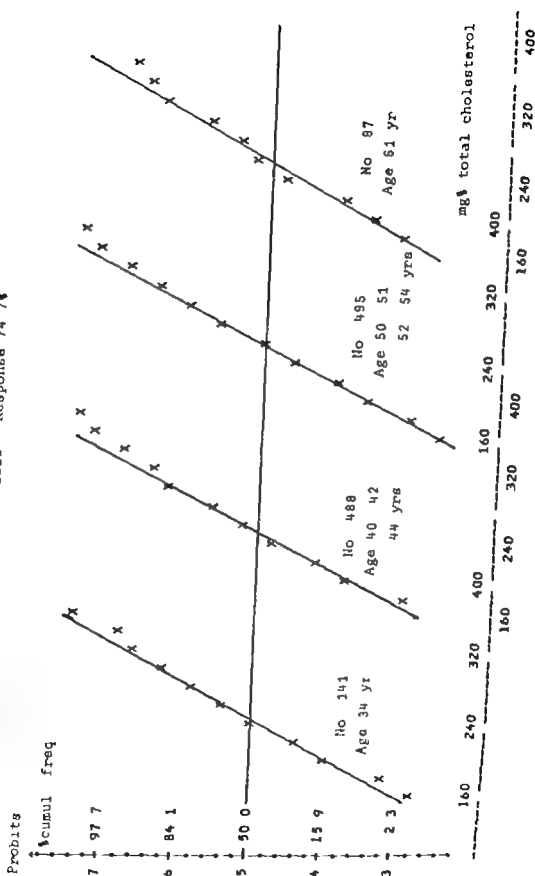
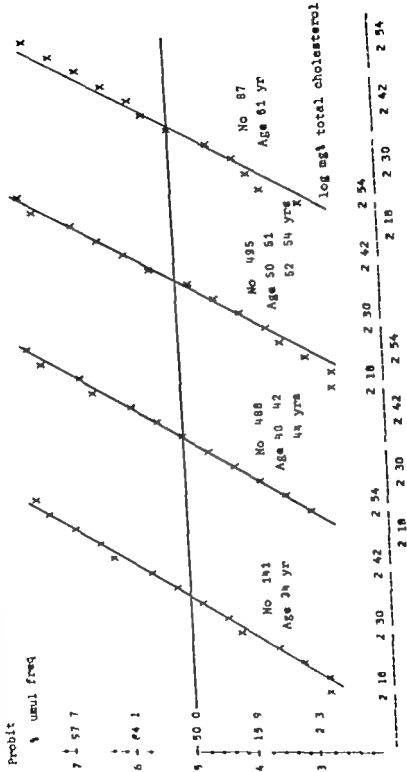
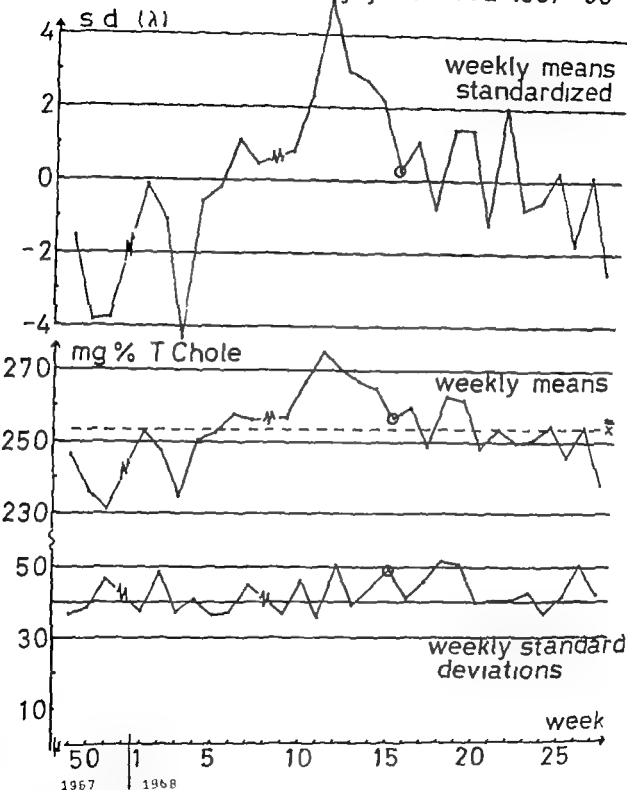


FIG. 1. A 111 13 health survey in the Reykjavik area Stage I 1967 88 M n
 Fractil diagrams of the distribution of the logarithm of serum total cholesterol values in men
 Total number (No.) in the four cohorts 1211 Response 74.7%



Health survey in the Reykjavik area 1967-'68



Obs. pr week 45-86 except (O) 20 (M) none

Figure A III 14 Health survey in the Reykjavik area Stage I 1967-68 Men Weekly mean values (\bar{x}) weekly standard deviation (s) and weekly mean values standardized ($\lambda = (\bar{x} - \bar{x}) / s$) of serum total cholesterol values in men $n = \text{cts pr week}$ \bar{x} grand mean

period 240 mg% and 246 mg% respectively (see also the Discussion)

Triglycerides.

Analyses for triglycerides were started in the middle of January 1968 and were continued throughout the period of the survey. Triglyceride content was measured in the serum of 1787 participants or from 81.1% of those who attended the clinic; the contributions of different age groups being very similar, varying between 78% - 85% (Table A III 12). The results are expressed as mg% (mg/100 ml) to an accuracy of 1 mg%.

From the fractile diagrams and the histograms the results from each cohort do not appear to be normally distributed (Figs A III 15-16). The distributions are very skew, tailing more to right. This is also evident from Fig. A III 18 which shows the mean value to be some 10 mg% higher than the median values and a 2-4 times greater difference between the 90% - 95% fractiles than the 5% - 10% fractile.

All the coefficients of variation are about 0.5. The logarithm of the results is normally distributed to a very good approximation as shown by the fractile diagrams (Fig. A III 17).

The mean value, median values and the lower fractiles all seem to be to a good approximation age independent at any rate for the 40 to 60 year old age groups. Thus the mean values in these age groups are about 95 mg%, the median values about 85 mg%, and the 10% and 5% fractiles are a little less than 50 mg% and still more than 35 mg% respectively.

The same does not apply to the higher fractiles. These show a clear increase from the age of 34 up to 50 years but then appear to decrease again. Thus the 95% fractile increases from about 160 mg% for 35 year old men to 210 mg% over the next 15 years, an increase of some 3 mg% per year. This is followed by a decrease of some 20 mg% from 50 to 60 years, i.e. some 3 mg% per year. The position of the 90% fractile is somewhat smaller; the increase over the first 15 years is about 30 mg% and the decrease over the following 10 years appears to be somewhat

Table A III 12 Health survey in the Reykjavik area Stage I 1967 - 88 - Men
Frequency table of serum triglyceride values in men Total response 75 1¹/

Values in mg/100ml (mg%)	Class width 20 mg%	Age in years														All age groups	
		37	40	42	44	46	47	48	49	50	51	52	54	56	58		61
5	0	1	0	1	1	3	0	1	0	0	0	1	0	0	0	0	5
18	0	1	1	1	4	0	2	1	0	3	0	1	0	1	0	1	18
61	0	1	2	5	7	3	7	4	8	2	0	2	6	1	1	4	61
131	0	1	12	15	17	15	12	7	8	5	0	8	11	6	7	3	131
184	0	1	13	11	15	18	11	11	7	11	13	10	11	10	12	7	184
206	0	1	23	11	1	16	21	21	12	9	17	16	9	12	7	11	206
239	0	1	16	20	13	19	15	18	18	9	17	11	17	20	14	12	239
181	0	1	14	18	15	15	13	13	6	9	11	13	8	7	5	8	181
147	0	1	15	11	17	13	10	11	8	9	13	8	8	9	6	2	147
125	0	1	11	8	3	12	7	12	7	8	3	4	6	7	5	3	125
78	0	1	9	6	5	5	6	4	3	5	5	2	9	7	2	3	78
88	0	1	7	7	11	7	7	6	6	1	3	5	3	3	1	5	88
66	0	1	2	4	4	4	3	7	0	4	4	3	4	4	3	3	66
49	0	1	5	4	3	3	5	5	4	3	0	2	3	3	1	2	49
37	0	1	4	1	1	3	2	2	2	2	0	3	5	0	1	1	37
26	0	1	3	4	2	0	2	3	2	2	2	2	0	1	0	2	26
31	0	1	3	4	2	1	3	3	2	2	2	0	1	1	0	0	31
22	0	1	2	4	1	1	3	1	2	2	2	0	1	2	1	0	22
19	0	1	1	1	2	0	0	0	0	0	0	2	1	1	0	2	19
13	0	1	1	1	1	0	0	2	0	0	1	0	0	1	0	0	13
11	0	1	0	0	0	1	2	1	1	0	1	0	1	0	0	1	11
10	0	1	0	0	1	1	1	1	1	0	1	1	0	0	0	1	10
7	0	1	0	1	0	2	2	2	1	1	1	0	0	0	0	1	7
4	0	1	4	2	1	3	3	4	3	2	1	2	1	0	0	0	4
29	0	1	15	13	14	12	14	14	10	9	10	7	10	1	1	0	29
Total ()		141	135	134	140	122	147	140	100	91	103	96	105	97	70	71	1787
Man % of no Investigated		141	135	134	140	122	147	140	100	91	103	96	105	97	70	71	1787
1/ 1/ Investigated		141	135	134	140	122	147	140	100	91	103	96	105	97	70	71	1787
68		141	135	134	140	122	147	140	100	91	103	96	105	97	70	71	1787
1/ 1/ Investigated		141	135	134	140	122	147	140	100	91	103	96	105	97	70	71	1787

stage I started in Nov 67 but the triglyceride measurements began in January
Rec use of the appointment system the 1987 men can be considered as a stratified random
apl of those investigated

1/ 1/ Investigated stage I started in Nov 67 but the triglyceride measurements began in January 68. Because of the appointment system the 1787 men can be considered as a stratified random sample of those investigated.

Fig A III 19 p 61 shows graphs of the means (\bar{X}) and standard deviations (s) for each week throughout the period from the middle of January 1968 up to the beginning of the summer vacation in the beginning of July. Also shown is a graph of the standardized weekly deviations (λ) from the grand mean (\bar{X}). These deviations were computer calculated according to the formula $\lambda = (\bar{X} - \bar{X}) / (s/\sqrt{n})$ where s is the standard deviation per week and n is the number of results per week. The standard deviations appear to be steady throughout the period fluctuating around a value of a little less than 50 mg/l. The standard deviations of the weekly means vary between 3.6 and 12.5 mg/l (most between 5.0 and 8.5 mg/l). There is no apparent systematic variation in the results over the period.

Table A III 13

Health survey in the Reykjavik area Stage I 1967 - 68 1st
 Cumulative distributions of serum triglyceride values in men
 Total response 75 1^{1/}

Triglycerides values in mg/100 ml (mg%)	Age in years						
	34	37	40 42 44	46 47 48 49	50 51 52 54	56 58	61
< 20	8	0	5	2	1	0	0
- 30	25	8	20	1	13	6	14
- 40	85	59	44	51	18	18	70
- 50	221	149	105	114	115	96	113
- 60	373	231	184	214	210	228	211
- 70	483	322	98	334	344	341	366
- 80	595	430	408	473	474	545	515
- 90	678	554	523	568	579	617	648
- 100	754	603	628	639	668	707	676
- 110	814	686	694	717	735	778	718
- 120	822	736	743	752	788	832	761
- 130	841	785	807	807	819	856	831
- 140	941	813	836	817	857	898	873
- 150	947	893	865	964	878	934	901
- 160	947	901	890	892	903	940	915
- 170	953	934	912	904	911	946	944
- 180	975	953	934	923	926	952	944
- 190	991	959	951	937	934	970	944
- 200	991	975	966	941	947	976	972
- 210	991	975	978	947	959	982	972
- 220	991	975	978	961	967	984	972
- 230	991	975	97	970	977	998	945
- 240	991	975	980	976	997	998	1000
- 250	991	975	981	983	99	998	1000
- 260	991	97	983	987	997	998	1000
- 270	991	975	983	983	997	998	1000
- 280	991	975	985	990	995	998	1000
- 290	991	983	988	99	995	994	1000
No. of respondents (N)	115	111	404	509	332	167	71
N as % of no. investigated	33.7	41	83	91	79.1	73.3	81

1/ 1/2 investigated in Stage I started in Nov. 67 but the 100% response rate began in January 68 because of the appointment of the 1787 men in the 1st round as a stratified random sample of the investigated population.

Table A III 14

Health survey in the Reykjavik area Stage I 196 - 68 - Men
 Numerical characteristics of the distributions of serum triglyceride
 values in men : Total reasons 75 1^{1/}

Triglyceride values in mg/100ml(mg%)	Age in years						
	34	37	40 42 44	46 47 48 49	50 51 52 54	56 58	61
Mean (%)	82 11	97 73	100 17	98 73	96 27	90 86	92 52
Variance	2772 90	2372 12	2589 72	2834 34	2702 30	1832 04	1963 34
s d (%)	42 10	50 71	50 88	53 23	51 98	42 80	44 30
s/z	51	52	51	54	54	47	48
2/ 5 % centile	32 92	36 44	37 61	38 44	37 80	40 30	37 78
10 %	37 83	43 54	49 30	47 87	47 60	50 84	47 93
20 %	47 67	56 40	60 32	58 32	57 92	58 99	57 39
30 %	56 10	67 93	70 65	67 41	66 63	67 13	65 28
40 %	63 97	77 21	79 54	76 19	75 24	74 76	72 90
50 %	72 24	85 86	88 43	84 82	83 76	82 02	80 00
60 %	82 50	97 50	99 47	95 02	93 19	89 28	87 10
70 %	93 75	113 33	111 50	108 24	106 04	100 80	105 30
80 %	108 50	133 27	129 28	128 85	126 01	116 04	125 00
90 %	136 00	154 10	165 34	168 60	164 04	141 73	150 10
95 %	152 50	183 50	189 94	213 08	203 30	173 75	195 00
Min	13 00	25 00	13 00	10 00	18 00	30 00	28 00
Max	320 00	310 00	360 00	450 00	510 00	337 00	238 00
Range	305 00	285 00	347 00	440 00	492 00	307 00	210 00
No of measures (N)	118	121	409	509	392	167	71
% of no in stig ted	83 7	81 2	83 3	81 4	79 0	78 0	81 6

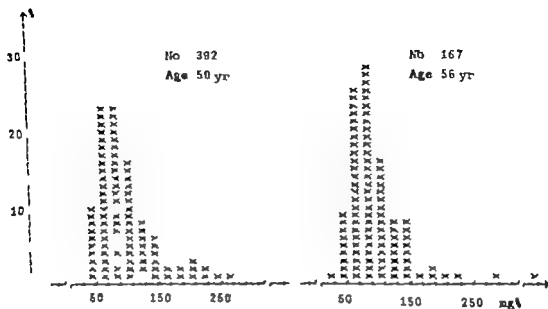
- 1/ % investigated Stage I started in low 67 but the triglyceride
 measurements began in January 68 Because of the appointment
 system the 1967 result can be considered as a stratified random
 sample of those investigated
- 2/ Cutting points below which are found 5 10 20 etc per cent
 of the values

Table A III 13

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Cumulative distributions of serum triglyceride values in men
 Total response 75 1/2

Triglycerides values in mg/100 ml (mg%)	Age in years						
	34	37	40 47 44	46 47 48 49	50 51 5 54	56 58	61
≤ 20	8	0	5	2	3	0	0
~ 30	2 5	8	2 0	3	1 3	6	1 4
~ 40	8 5	5 9	4 4	5 1	3 8	1 8	7 0
~ 50	22 3	14 9	10 5	11 4	11 5	9 6	11 3
~ 60	37 3	23 1	18 6	21 4	23 0	22 8	21 1
~ 70	48 3	32 7	29 8	33 4	34 4	34 1	36 6
~ 80	59 5	43 0	40 8	47 3	47 4	54 5	53 5
~ 90	67 8	55 4	52 3	56 6	57 9	61 7	64 5
~ 100	75 4	60 3	62 8	63 9	66 8	70 7	67 6
~ 110	81 4	68 6	69 4	71 7	73 5	77 8	71 8
~ 120	87 2	73 6	74 3	75 2	78 8	83 2	76 1
~ 130	88 1	78 5	80 7	80 7	81 9	85 6	83 1
~ 140	94 1	84 3	83 6	83 7	85 7	89 8	87 3
~ 150	94 3	89 3	85 5	86 4	87 6	93 4	90 1
~ 160	94 3	90 1	89 0	89 2	90 3	94 0	91 5
~ 170	95 8	93 4	91 7	90 4	91 1	94 6	94 4
~ 180	97	95 9	93 4	92 1	92 6	95 2	94 4
~ 190	99 1	95 9	95 1	93 7	93 4	97 0	94 4
~ 200	99 1	97 5	96 6	94 1	94 7	97 6	97 2
~ 210	99 1	97 5	97 8	94 7	95 7	98 2	97 2
~ 220	99 1	97 5	97 8	96 1	96 7	98 8	97 2
~ 230	99 1	97 5	97 8	97 0	97 7	98 8	98 5
~ 240	99 1	97 5	98 0	97 6	98 7	98 8	100 0
~ 250	99 1	97 5	98	98 1	98 5	98 8	100 0
~ 260	99 1	97	98 3	98 3	98 7	98 8	100 0
~ 270	99 1	97 5	98 3	98 3	99 2	98 8	100 0
~ 280	99 1	97 5	98 5	99 0	97 5	98 8	100 0
~ 290	99 1	98 3	98 8	99	97 5	97 4	100 0
No of measurements (N)	115	121	409	67	312	167	71
N as % of no investigated	43 2	41 2	81	91 4	79 3	73 0	81 4

1/ % investigated Stage I started in Nov 67 but the TG measurements began in January 68. Because of the appointment system the 1787 men can be considered as a stratified random sample of those investigated.



Class width 20 mg/l

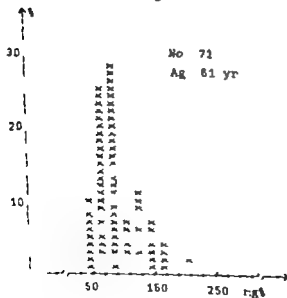
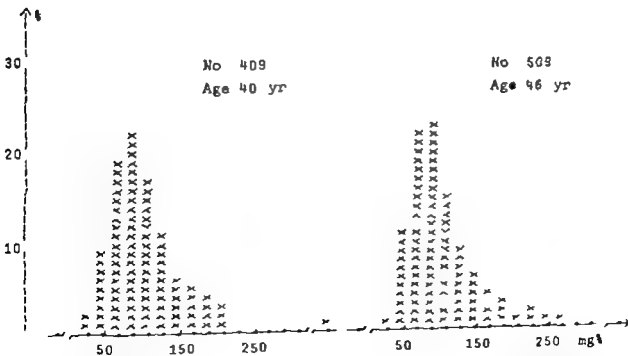
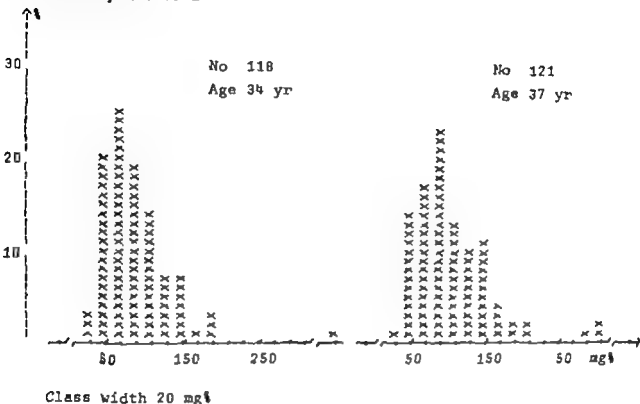


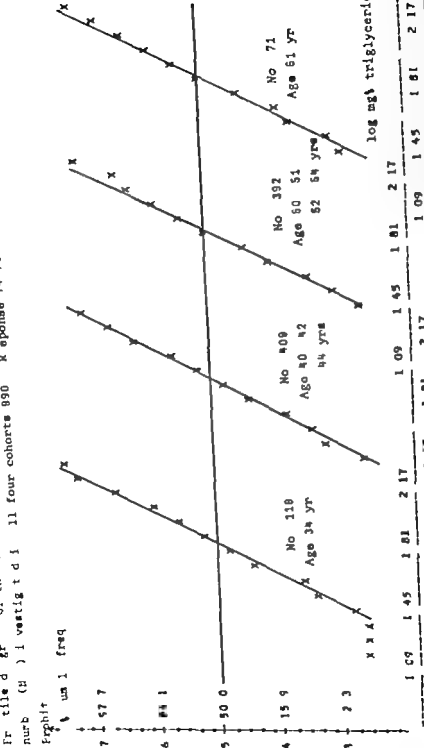
Figure A III 15

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Histograms showing distributions of serum triglyceride values in men
 Total response 75 1%



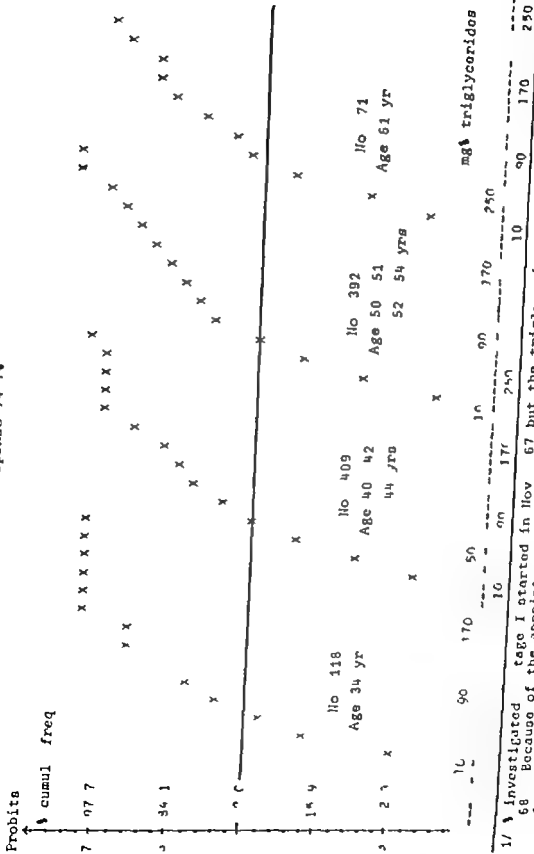
1/ % investigated Stage I started in Nov 67 but the triglyceride measurements began in January 68. Because of the appointment system the 1787 men can be considered as a stratified random sample of those investigated

Figure A III 17. 1. Plot of \log triglyceride values in men Total
 of the distribution of \log triglyceride values in men Total
 for the distribution of \log triglyceride values in men Total



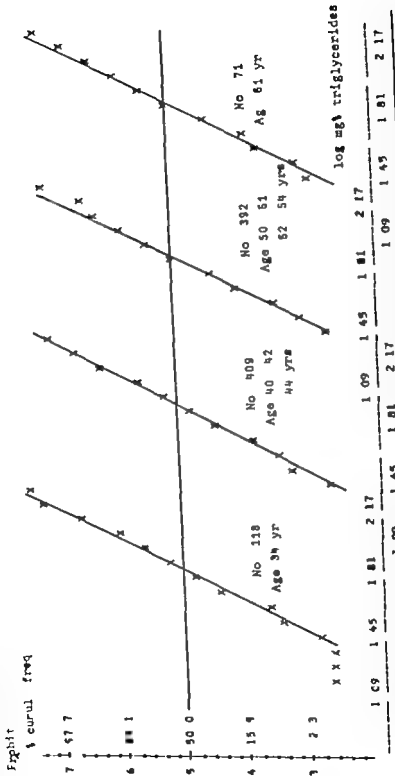
1/ The investigation started in Nov 67 but the triglyceride measurements began in January 68. Because of the appointment system the 990 men can be considered as a stratified random sample of those investigated.

health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Fracile diagrams of the distributions of serum triglyceride values in men Total number (No)
 investigated in all four cohorts 990 Response 74 7^{1/}



1/ Investigated stage I started in Nov 67 but the triglyceride measurements began in January 68. Because of the appointment system the 990 men can be considered as a stratified random sample of those investigated

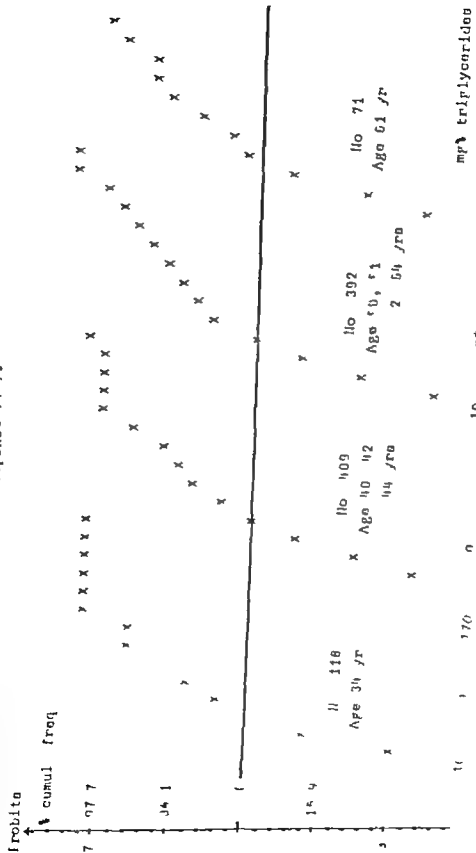
Fig 10 A III 17. h lth s y in th Resy k j ik area St ge I 1967 Men
 Fractil dis s f th d trib ti ns of th log rithms of s rum trigly rid values in men Total
 number (No) in t gnt d = 11 f ur cohorts 990 Respons 74 78 1/



1/ 1 investigated Stage I started in Nov 67 but the triglyceride measurements began in January 68. Because of the appointment system the 990 men can be considered as a stratified random sample of those investigated

Figure A III 15

Health survey in the Reykjavik area Stage I, 1967 - 68 - Men
 Fractile diagrams of the distributions of serum triglyceride values in men Total number (No.)
 investigated in all four cohorts 990 Response 74 71/



mp triglycerides
 1/ If you start in 1967 but the triglyceride measurements taken in January
 1968 are for the type I diet system then 990 men can be considered as a stratified random sample

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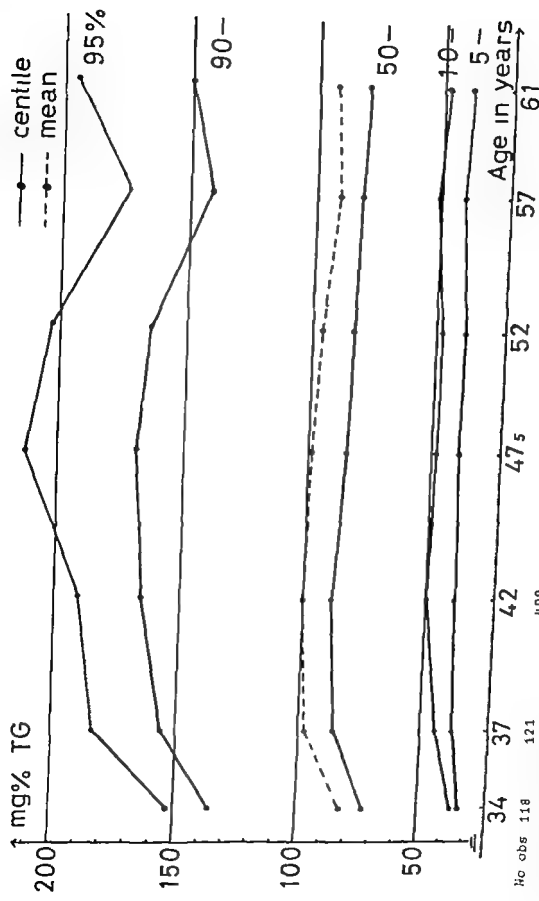
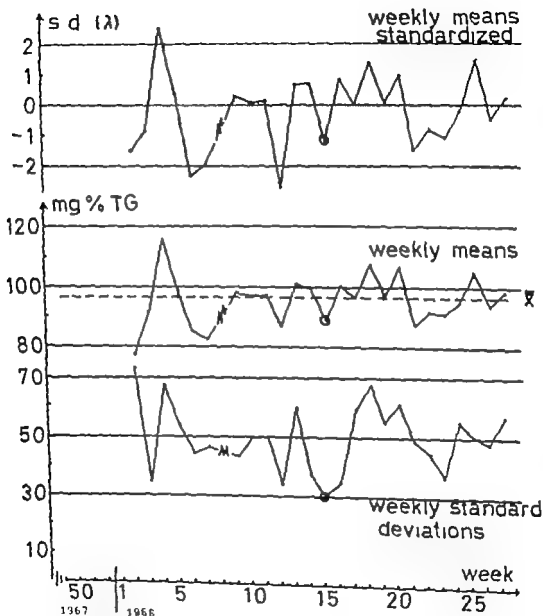


Figure A III 18 Health survey in the Reykjavik area
Diagram showing the mean, median and a few centiles of the distributions of serum triglyceride values in 7 cohorts (34 yr) (37 yr) (40 42 44 yrs) (46 47 48 49 yrs) (50 51 52 54 yrs) (56 58 yrs) and (61 yr)

Health survey in the Reykjavik area 1967-'68



to 5 weeks 32 86 except (o) 20 (M) none

for A III 19 1 alt) rvey in the Reykjavik area tag I 1967-
 s (a) kly rea v l (Σ) weekly standard deviations (Σ)
 nd 1/ r n alues standardized (λ) (Σ Δ) $\overline{m/s}$ of s run tri
 cl r d ($^{\circ}$ C) v lues in r n
 a no f obs pr week Σ = gr nd ean

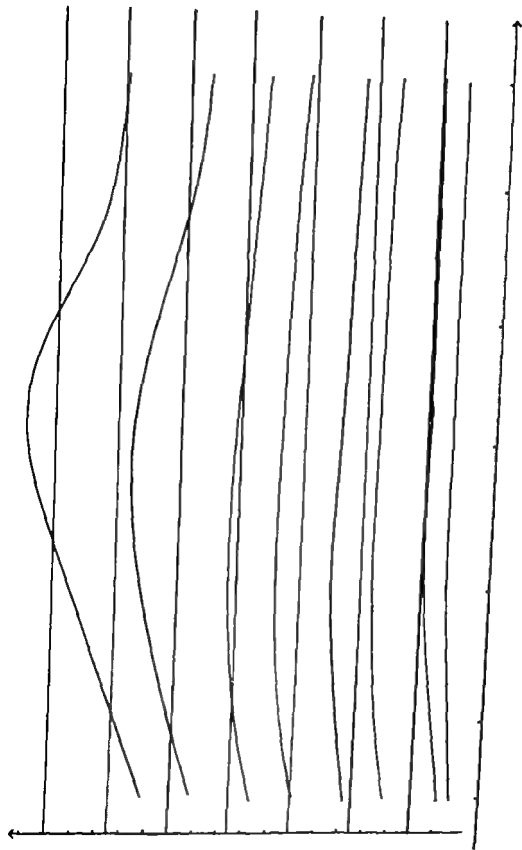


Figure A III 18b Lye-fitted curves (Obs highly subjective) 5 10 20 30 50 70 80 90 95 centiles

Beta lipoproteins, total cholesterol and triglyceride correlations

There is a positive correlation between values for beta-lipoproteins and total cholesterol (Tables A III 15 21). The correlation coefficients decrease with increasing age and their values are much the same whether or not the logarithms of the results are used. The correlation coefficients decrease from about 0.7 at the age of 34 to about 0.5 at the age of 51.

A correlation is also seen from Fig A III 21 (p. 54) which is based on the above mentioned tables. The figure shows how the means of the results of beta lipoprotein measurements increase with increasing total cholesterol concentration in the cohorts 40-44, 45-49 and 50-54 years. The results of the beta lipoprotein measurements in each of these cohorts were grouped according to the total cholesterol values and mean values for beta lipoproteins for each group calculated. The mean values appear to increase evenly or by about 0.09 mm IC per 10 mg% of total cholesterol at any rate up to a cholesterol value of 250 mg%. For higher cholesterol values the rate of increase in IC seems to be less.

A very similar correlation is seen for the results for beta lipoproteins and triglycerides (Tables A III 22 23). The correlation coefficients do however appear to decrease more with age or from about 0.7 to about 0.4. In Fig A III 22 it can be seen that the mean values for beta lipoproteins increase by about 0.09 mm IC per 10 mg% of triglycerides at any rate up to a triglyceride value of 120 mg%. For higher TG values there seems to be little if any increase in IC.

The correlation between total cholesterol and triglyceride values quite different. The correlation coefficients are positive but low or about 0.1 and appear to be virtually age independent. In Fig A III 23 it can be seen that the mean values for triglycerides increase almost linearly with increasing total cholesterol values or by about 4 mg% per 10 mg% of total cholesterol.

Table A III 36 p. 88 shows the partial correlation coefficients for the values of these three serum lipids. Here the partial correlation coefficients between total cholesterol and triglycerides appear to be none (zero).

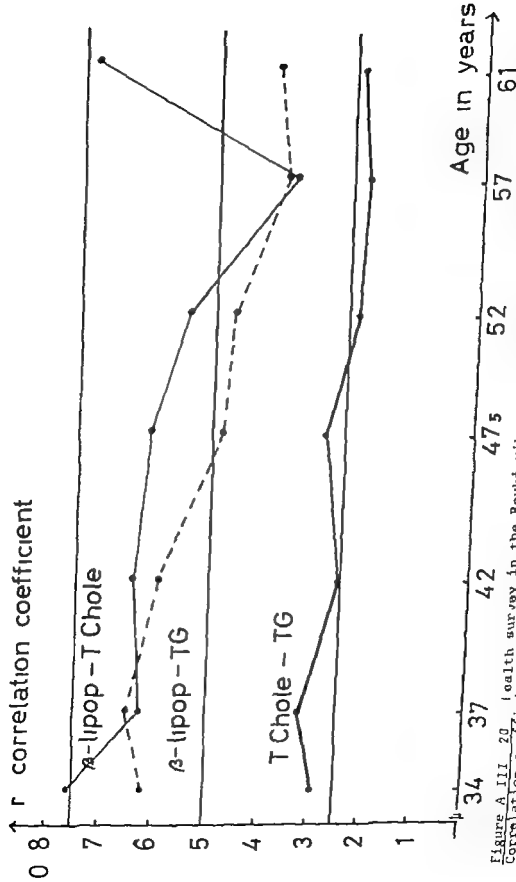


Figure A III 20 Health survey in the Reykjavik area Stage I 1967-68 - Men
Correlation coefficients of α -lipoprotein and total cholesterol and triglycerides plotted against mean age of 7 cohorts- (34yr) (40 yr) (44 yrs) (46 47 48 49 yrs) (50 52 54 yrs) (56 58 yrs) and (61 yr) - Based on Tables A III

Health survey in the Reykjavik area Stage I 1967-68 - Men.

mm Immunoc β -lipop

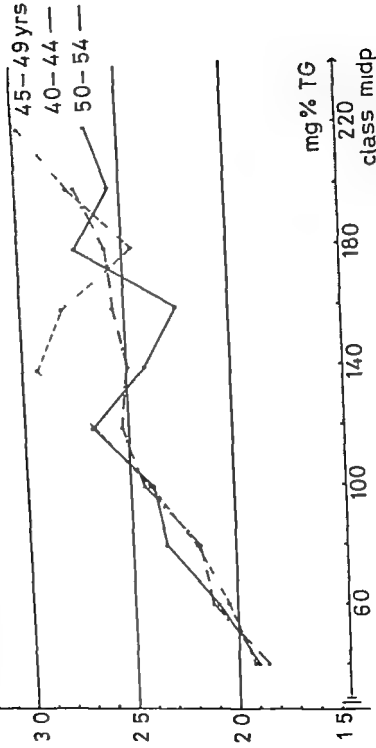


Figure A III 22 Health survey in the Reykjavik area Stage I 1967-68 - Men
Mean values of lipoproteins plotted against triglyceride values within three cohorts
Based on Tables A III 22 28

Health survey in the Reykjavik area Stage I 1967-'68 - Men

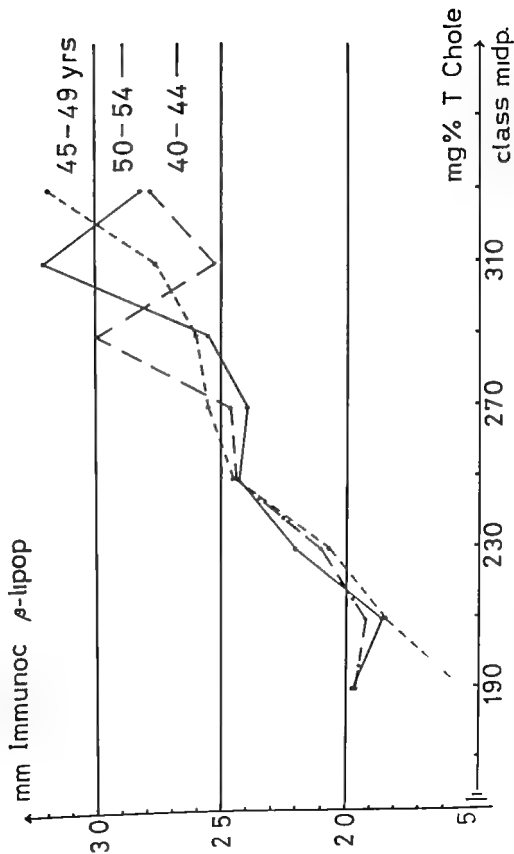


Figure A III 21. Health survey in the Reykjavik area Stage I 1967 - 68 - Men
Mean values of β -lipoproteins plotted against grouped total cholesterol values within three cohorts - Based on Tables A III 16-21

Health survey in the Reykjavik area Stage I 1967-68 - Men.

mm Immunoc β -lipop

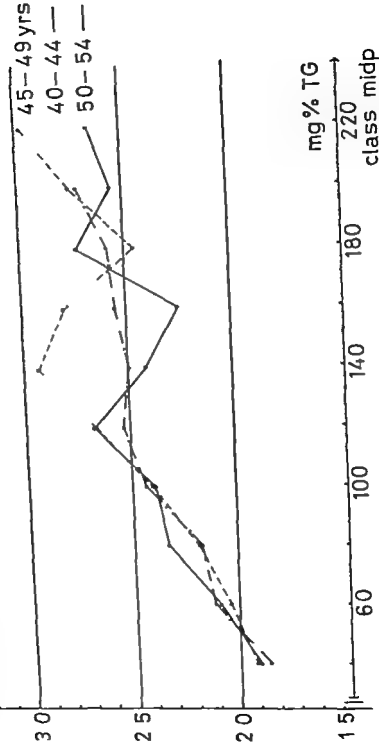


Figure A III 22 Health survey in the Reykjavik area Stage I 1967 - 68 Men
 An values of β lipoproteins plotted against grouped triglyceride values within three cohorts
 Based on Tables A III 22 28

Health survey in the Reykjavik area Stage I 1967-'68 - Men

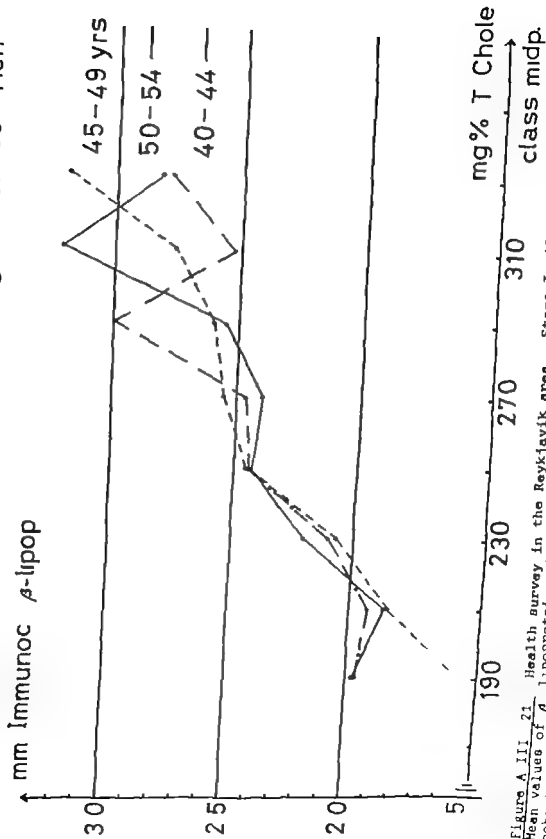


Figure A III 21 Health survey in the Reykjavik area Stage I 1967 - 68 - Men
Mean values of β lipoproteins plotted against grouped total cholesterol values within three cohorts Based on Tables A III 15-21

Health survey in the Reykjavik area Stage I, 1967-'68 - Men

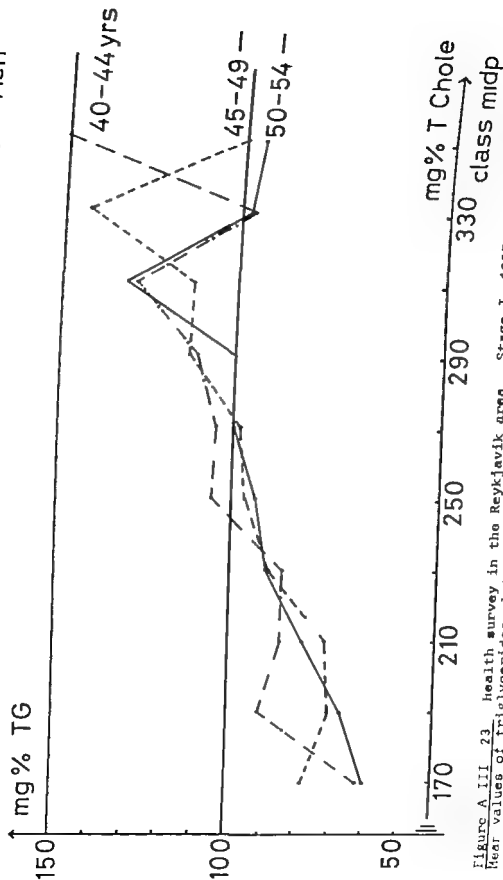


Figure A III 23 health survey in the Reykjavik area Stage I 1967 - 68 - Men
Mean values of triglycerides plotted against grouped total cholesterol values within three cohorts Based on Tables 29 35

Tbl	All 15	lith r y d th R y k j a l k r c.	Stag I 1907	68	M n
Frc q	y t l l f e rum B l p e p r o t	I s a n d s u m t o t a l c h o l e s t r o l			a l u s i n m e n
Ber f m	23	A g s e v R s p o a 71 6 %			

Table A III 17

Frequency	Number of years	Response	Percentage
1	1	1	16.4%
2	2	2	16.4%
3	3	3	16.4%
4	4	4	16.4%
5	5	5	16.4%
6	6	6	16.4%
7	7	7	16.4%
8	8	8	16.4%
9	9	9	16.4%
10	10	10	16.4%
11	11	11	16.4%
12	12	12	16.4%
13	13	13	16.4%
14	14	14	16.4%
15	15	15	16.4%
16	16	16	16.4%
17	17	17	16.4%
18	18	18	16.4%
19	19	19	16.4%
20	20	20	16.4%
21	21	21	16.4%
22	22	22	16.4%
23	23	23	16.4%
24	24	24	16.4%
25	25	25	16.4%
26	26	26	16.4%
27	27	27	16.4%
28	28	28	16.4%
29	29	29	16.4%
30	30	30	16.4%
31	31	31	16.4%
32	32	32	16.4%
33	33	33	16.4%
34	34	34	16.4%
35	35	35	16.4%
36	36	36	16.4%
37	37	37	16.4%
38	38	38	16.4%
39	39	39	16.4%
40	40	40	16.4%
41	41	41	16.4%
42	42	42	16.4%
43	43	43	16.4%
44	44	44	16.4%
45	45	45	16.4%
46	46	46	16.4%
47	47	47	16.4%
48	48	48	16.4%
49	49	49	16.4%
50	50	50	16.4%
51	51	51	16.4%
52	52	52	16.4%
53	53	53	16.4%
54	54	54	16.4%
55	55	55	16.4%
56	56	56	16.4%
57	57	57	16.4%
58	58	58	16.4%
59	59	59	16.4%
60	60	60	16.4%
61	61	61	16.4%
62	62	62	16.4%
63	63	63	16.4%
64	64	64	16.4%
65	65	65	16.4%
66	66	66	16.4%
67	67	67	16.4%
68	68	68	16.4%
69	69	69	16.4%
70	70	70	16.4%
71	71	71	16.4%
72	72	72	16.4%
73	73	73	16.4%
74	74	74	16.4%
75	75	75	16.4%
76	76	76	16.4%
77	77	77	16.4%
78	78	78	16.4%
79	79	79	16.4%
80	80	80	16.4%
81	81	81	16.4%
82	82	82	16.4%
83	83	83	16.4%
84	84	84	16.4%
85	85	85	16.4%
86	86	86	16.4%
87	87	87	16.4%
88	88	88	16.4%
89	89	89	16.4%
90	90	90	16.4%
91	91	91	16.4%
92	92	92	16.4%
93	93	93	16.4%
94	94	94	16.4%
95	95	95	16.4%
96	96	96	16.4%
97	97	97	16.4%
98	98	98	16.4%
99	99	99	16.4%
100			

Respond

1946 1947 1948

[illegible]

no : / zero + in value (x)

Correlation coefficient

... total values (y)

Correlation coefficient	Total cholesterol	LDL cholesterol	HDL cholesterol
0.75	247.15	124.71	122.44

mean log x: 349 d log x 0963 r(log x log y): 0.97

1/ % investigated Stage I started in Nov 1967 but the ρ lipoprotein measurements began in May 1968. Because of the pointment system the 146 men can be considered as a random sample of those investigated

Table A III 16 Health survey in the Reykjavik area Stage I 1967 - 68 - Men
Frequency table of serum B-lipoprotein values and serum total cholesterol values in men
Number of men 38 Age 37 years Response 71 3%^{1/}

Total cholesterol values in mg/100ml (mg%)									
B lipoprotein	150	170	190	210	230	250	270	290	310
Immunoc	150	170	190	210	230	250	270	290	310
Class midpoint minus 0.5 mg %	150	170	190	210	230	250	270	290	310
(Class width 20 mg %)	150	170	190	210	230	250	270	290	310
Σ	85	135	125	145	165	185	205	225	245
1			1						
2			1						
3			1						
4			1						
5			1						
6			1						
7			1						
8			1						
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93			1						
94			1						
95			1						
96			1						
97			1						
98			1						
99			1						
100			1						

B lipoprotein values (x)									
Mean x	Σ	Σ log x	Σ log x	Σ log x	Σ log x	Σ log x	Σ log x	Σ log x	Σ log x
Mean log x	Σ	Σ log x	Σ log x	Σ log x	Σ log x	Σ log x	Σ log x	Σ log x	Σ log x
Correlation coefficient	Σ	Σ log x	Σ log x	Σ log x	Σ log x	Σ log x	Σ log x	Σ log x	Σ log x
Total cholesterol values (y)	Σ	Σ log y	Σ log y	Σ log y	Σ log y	Σ log y	Σ log y	Σ log y	Σ log y
Mean y	251.89	Mean log y	2.397	Mean log y	2.397	Mean log y	2.397	Mean log y	2.397
S d y	35.85	S d log y	0.654	S d log y	0.654	S d log y	0.654	S d log y	0.654
1/ Investigated Stage I started 'r Nov 1967 but the β-lipoprotein measurements began in May 1968. Because of the appointment system the 38 men can be considered as a random sample of those investigated									

Table A III 17. In the R y k j a i k a r a Stage I 1967 holost rol values in men

freq on y tab1 f serum B l poprot i l u s and serum total holost rol values in men

Number f n 146 Ag 40 42 44 y are Respon 76 41/

Total hol sterol value s in mg/100ml (mg%)

B lipop men	Class midpoint minus 0.5 % (Class width 20 mg %)										
	150	170	190	210	230	250	270	290	310	330	350
1	1	2	3	7	3	4	1	1	2	1	1
1.05											
1.15											
1.25											
1.35											
1.45											
1.55											
1.65											
1.75											
1.85											
1.95											
2.05											
2.15											
2.25											
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3.55											
3.65											
3.75											
3.85											
3.95											
4.05											
4.15											
4.25											
4.35											
4.45											
4.55											

Total cholesterol values (y)

B lipoprotein values (x)	Correlation coefficient										
	2 29	S d x	549	r(x,y)	6426	Mean y	247 15	S d y	45 28	Mean log y	384 15
Mean x	2 29	S d x	549	r(x,y)	6426	Mean y	247 15	S d y	45 28	Mean log y	384 15
Mean log x	349	S d log x	0963	r(log x log y)	6470	Mean log y	384 15	S d log y	0769		

1/ A investigated Stage I started in Nov 1967 but the A lipoprotein measurements began in May 1968. Because of the appointment system the 148 men can be considered as a random sample of those investigated

T bl A III 19 il lth ur ey in the R ykja ik are St g I 1967 Men
 Freq cy t bl f a run B l poprot in value and a run total cholesterol valu s in m n
 Number of n 118 Age 50 51 52 54 y are Response 75 7% 1/

Total cholest rol valu s in mg/100ml (mg%)

(Class width 20 mg %)

B lipop mm	Class midpoint minus 0 5 mg %										
	150	170	190	210	230	250	270	290	310	330	350
1 03											
1 25											
1 45											
1 65											
1 85											
2 05											
2 25											
2 45											
2 65											
2 85											
3 05											
3 25											
3 45											

CL midp	Class midpoint minus 0 5 mg %										
	150	170	190	210	230	250	270	290	310	330	350
85											
1 03											
1 25											
1 45											
1 65											
1 85											
2 05											
2 25											
2 45											
2 65											
2 85											
3 05											
3 25											
3 45											

B lipoprotein values (x)	Correlation coefficient										
	150	170	190	210	230	250	270	290	310	330	350
85											
1 03											
1 25											
1 45											
1 65											
1 85											
2 05											
2 25											
2 45											
2 65											
2 85											
3 05											
3 25											
3 45											

B lipoprotein values (y)	Total cholesterol values (y)										
	150	170	190	210	230	250	270	290	310	330	350
85											
1 03											
1 25											
1 45											
1 65											
1 85											
2 05											
2 25											
2 45											
2 65											
2 85											
3 05											
3 25											
3 45											

Mean x : 2 30 S d x 532 r(x y) : 5304 Mean y : 248 19 S d y 38 78
 Mean log x : 350 d log x : 0941 r(log x log y) : 6106 Mean log y : 2 370 S d log y 0669
 1/ I investigated tage I started in Nov 1968 but the lipoprotein measurement started in May 1988 Because of the appointment system the 116 men can be considered as a random sample of those investigated

Table A III 71. Health record of R. J. Javik, a Stage I 1967 88 Men
 Frequency of blood lipoprotein in all and serum total cholesterol values in men
 Labor force 19 Age 61 years Response 69.6%^{1/}

Total cholesterol values in mg/100ml (mg%)

B lipoprotein (Class width 20 mg %)

Frequency of blood lipoprotein in all and serum total cholesterol values in men

Class midpoint minus 0.5 mg % (Class width 20 mg %)

Frequency of blood lipoprotein in all and serum total cholesterol values in men

Frequency of blood lipoprotein in all and serum total cholesterol values in men

Frequency of blood lipoprotein in all and serum total cholesterol values in men

Frequency of blood lipoprotein in all and serum total cholesterol values in men

Frequency of blood lipoprotein in all and serum total cholesterol values in men

Frequency of blood lipoprotein in all and serum total cholesterol values in men

Frequency of blood lipoprotein in all and serum total cholesterol values in men

Frequency of blood lipoprotein in all and serum total cholesterol values in men

Frequency of blood lipoprotein in all and serum total cholesterol values in men

Frequency of blood lipoprotein in all and serum total cholesterol values in men

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Frequency of blood lipoprotein in all and serum total cholesterol values in men

Frequency of blood lipoprotein in all and serum total cholesterol values in men

Frequency of blood lipoprotein in all and serum total cholesterol values in men

B lipoprotein values (x) Correlation coefficient Total cholesterol values (y)

Mean x : 204 S d : 257 r(x, y) : 7282 Mean y : 230.42 S d y : 36.63

Mean log x : 0.995 r(log x, log y) : 7562 Mean log y : 2.374 S d log y : 0.663

^{1/} Investigator Stage I started in Nov 1967 but the lipoprotein measurements began in May 1968. Because of the appointment system the 19 men can be considered as a random sample of those in estigated

Table A III 22 health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Frequency table of serum B-lipoprotein values and serum triglyceride values in men
 Number of men 33 Age 34 years Response 71 6 1/

		Triglyceride values in mg/100ml (mg%)															
		Class midpoint minus 0.5 mg % (Class width 20 mg %)															
C1 midp		320	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
5	55		1														
1	05																
1	25																
1	45																
1	65			1													
1	85		4	3			1										
2	05	1	1	1													
2	25		2		1	1		1		1							
2	45			1	1												
2	65						1										
2	85							1									
3	05			1	1												
3	25																
3	45																
3	65																
3	85																
4	05																
4	25																
4	45																
4	65																

B lipoprotein values (x)

Mean x

Mean log x

2 22

S d x

333

d

log x

533

1127

r(x, y)

Correlation coefficient

6207

5586

Mean log y

91 79

S d y

1 891

S d log y

59 71

2465

Triglyceride values (y)

Mean y

Mean log y

S d y

S d log y

59 71

2465

1/

investigated

Stage I started in Nov 1967 but the 33 men can be considered as a random sample of those investigated

Because of the appointment system the 33 men can be considered as a random sample of those investigated

1968

Because of the appointment system the 33 men can be considered as a random sample of those investigated

1968

Because of the appointment system the 33 men can be considered as a random sample of those investigated

1968

[bl A III 21] 1 lth eur y th R ykj vik r Stag I 1987 88 Men
 Feq n y t bl of rum B lipoprot in alu s and s rum triglyc rid values in men
 Number f men 18 Ag 37 years R sponse 71 3% 1/

B lipop r Immunoc Cl midp	Triglyc rid alues in mg/100ml (mg%)															
	Class midpoint minus 0.5 mg % (Class width 20 = 10 %)															
	120	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
85				1												
105																
125																
145																
165																
185																
205																
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285																
305																
325																
345																
365																
385																
405																
425																
445																
465																

B-lipoprot in val u s (x)
 Mean x : 2 30 S d x : 684 r(x y) : 6916 Mean y : 116 68 S d y : 68 85
 H an log x : 361 S d log x : 1243 r(log x log y) : 6610 Mean log y : 2 003 S d log y : 2353
 1/ 1/ 1/ investigated Stage I started in Nov 1987 but the 1/ lipoprotein measurements began in May
 1988 Because of the appointment system the 38 men can be considered as a random sample of
 those investigated

[Bl A III 21] 1 lth sur y 1 th R ykj ik re St H^o I 1967 68 Men
 freq n y t bl of s run B-lipoprotein v lu and serum triglyceride valu e in men
 Number of no 18 Ag 37 year Response 71 31 2/

B lipop		Triglycerid value in mg/100ml (mg%)															
Immuno		Class midpoint min 0.5 mg % (Class width 20 mg %)															
Cl midp		20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
5	85				1												
	1 05																
	1 25																
	1 45	2															
	1 65	1															
	1 85	?															
	2 05	?															
	2 25	1															
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	4 45																
	4 65																

lipoprotein values (x) Correlation coefficient Triglyceride values (y)
 n x 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
 mean log x 361 S d log v 1243 r(log = log y): 6516 Mean y 116 68 S d y 1 MM 85
 1/ 1 investigated Stage 1 started in Nov 1967 but th e lipoprotein measurements began in May
 1968 Because of the appointment system the 38 men can b considered as a random sample of
 those inv stigat d

Health survey in the Reykjavik area

Frequency table of serum B-lipoprotein values and serum triglyceride values in men
Number of men 146 Age 40 42 44 years Response 75 48/

[illegible]

lipoprotein values (x)

mean x	7 20	5 d	3	Correlation coefficient
lean x				
...				

Triglyceride values (y)

	Mean log x	d log x	r(log x)	r(x y)	Mean y	Mean log y	S d y	S d log y
1/	349	0963	5978	5948	99 83	99 83	50 62	50 62

17. Stage I started in Nov 1967 but the β -lipoprotein measurements began in May 1968. Because of the appointment system the 146 men can be considered as those investigated

those investigated because of the appointment system the 146 men can be considered as a random sample of 1000 men over 100, but the β -lipoprotein measurements began in May

A II
 1 q y t h i f 159 A_r I t P y l j k r e t g e I 1967 68 H n
 b l p e p r o t n l n d s r u m t r i g l y c e r i d v a l u e s i n m o n
 K p o n 77 3¹/₁

I g l y l d l u s l m g / 100 m l (m g %)

(l n d p o n t m i s 0.5 m g % (C l a s s w i d t h 20 m g %)

2 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320

1

2

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11

12

13

14

15

16

correlation coefficient

r(x y)

r(log x log y)

Mean y

Mean log y

Triglyceride values (y)

Mean y : 95.77

Mean log y : 1.931

Sd

log y

49.57

205A

1/ A in otigat d tago I started in Nov 1967 but the A lipoprotein measurements began in May 1968. Because of the appointment system th 159 men can be considered as a random sample of those investigated

frequency table of serum β -lipoprotein values and serum triglyceride values in men
Number of men 146 Age 40 42 44 years Response 75 48^{1/}

[illegible]

```

--protein values (x)

```

Mean \bar{x}	Mean $\log x$	S.D.	$r(x, y)$	Correlation coefficient
2.29	2.40	0.31	0.549	0.549

Triglyceride values (y)

n y	99 83	S d	y		50 62
n log y	1 253	S d	log	:	

1/	investigated	Stage I started in Nov 1967	but the Δ lipoprotein measurements began in May 1968	Because of the appointment system the 146 men can be considered as a random sample of those investigated
		$\log x$	$0.963 \log y$	$2.918 \log y$
			5948	99 833
			Mean $\log y$	1 953
			$\log y$	$\log y$
				50 62

[illegible]

	freq	yr	bl	ul	AE
hurbs	f	42			

| Triglycerid v lue | In mg/100ml | (mg/l) |

Elipop (Class width 20 mg)

	1	midpoint	since	of	the	total
100	100	120	140	160	180	200

025 40 00

100

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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98	
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100	

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2
1

100

[illegible]

Population	\bar{x}	\bar{y}	$\bar{r}(x, y)$	$\log x$	$\log y$
1	2.76	14.9	516		
2	2.76	14.9	0931		

1/ I in outgated Stage I started in Nov 1987 but the

1960 Because of the appointment system the %
those investigated

Table A III 26 Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Frequency table of serum B-lipoprotein values and serum triglyceride values in men
 Number of men 114 Age 50 51 52 54 years Response 75 7^{1/}

B-lipoprotein	Triglyceride values in mg/100ml (mg%)												
	Class midpoint minus 0.5 mg % (Class width 20 mg %)												
mm	≤ 20	40	60	80	100	120	140	160	180	200	220	240	260 300 ≥ 320
85													
1 05													
1 25													
1 45													
1 65													
1 85													
2 05													
2 25													
2 45													
2 65													
2 85													
3 05													
3 25													
3 45													
3 65													
3 85													
4 05													
4 25													
4 45													
4 65													

B lipoprotein values (x)

Correlation coefficient

Triglyceride values (y)

lean x 2 7 S d x 467 r(x y)

Mean log x 347 S d log x 0873 r(log x log y)

4869

Mean y

93 35

S d y

47 68

1/ % investigated tag e I started in Nov 1967 but the B-lipoprotein measurements began in May 1968

Because of the appointment system the 114 men can be considered as a random sample of

t) e investigated

Tbl A.III.23 H lth e r e y n th R y k j a l k a r e Stage I 1987 E E M n
 f r e q y t b l f e r u m t o t a l c h o l e r o l v l u e s a n d s e r u m t r i g l y c e r i d e v a l u e s i n m e n
 n u m b e r o f m 118 A g 34 y a r s R e s p o n s e 71 g l /

Triglyceride values in mg/100 ml (mg%)

Tot chol mg %	Triglyc rid values in -----															
	Class midpt int minus 0.5 mg % (Class width 20 mg %)															
Cl midp	520	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
510.5			1			1	1		1							
170.5	2	1			2	1	2		1							
190.5		3	2	3	1	2	1									
210.5		2	2	7												
230.5		2	0													
250.5	1	8	3	4	5	3	3		2							1
270.5		2	2	3	3	1	1									
290.5		2	3	2	1											
310.5		1	1	2	1											
330.5				1		1										
350.5			1	1	1											
370.5																
390.5																
410.5																
430.5																
2450.5																

Triglyceride values (y)

Total cholesterol values (x)		Correlation coefficient		Triglyceride values (y)			
Mean x	244.00	Sd x	43.014	Mean y	82.11	Sd y	42.11
Mean log x	5.482	Sd log x	1.755	Mean log y	4.298	Sd log y	4.680

1/ I investigated Stage I start d in Nov 67 but the triglyceride measurements began in January
 B cause of the ppointment system the 118 men can be considered as a random sample of
 those investigated

Table A III 28 Health survey in the Reykjavik area Stage I 1967 - 68 ~ Men
Frequency table of serum B-lipoprotein values and serum triglyceride values in men
Number of men 19 Age 61 years Response 69 61/

[illegible]

3-lipoprotein values (x)

Mean x	Mean log x	Q	S	d	x	Correlation coefficient	Triglyceride values (y)			
		106	106	106	log x	r(x y)	Mean y	S	d	y
1/						r(log x log y)	Mean log y	S	d	log y
						0.595	397.6	74	68	20
						0.595	1.957	1.957	1.957	1.957

Stage I started in Nov 1967 but the β -lipoprotein measurements began in May 1968. Because of the appointment system the 19 man can be considered as a random sample of those investigated

Stag I 1987 68 Men

T bl A III 29 11 lth surv 3 1 the R ykjavik mg Stag I 1987 68 Men
 freq ncy t bl f serum total hol at rol alu and erum triglycerid values in men
 Number f me 118 Ag 34 y 6 R sponse 71 68 1/

Triglyc rid alues in mg/100 ml (mg%)

T t chole mg %	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
Cl midp	2	1	1	2	1	1	1	1	1							
5150 5																
170 5																
190 5																
210 5																
230 5																
250 5																
270 5																
290 5																
310 5																
330 5																
350 5																
370 5																
390 5																
410 5																
430 5																
450 5																

Triglyceride values (y)

Total cholest rol values (x)	Correlation coefficient	Mean y	Mean log y	S d	S d log y
Mean = 244 00 S d x 43 014 r(x y)	2965	42 11	4 298	280	468
Mean log x 5 482 S d log x 1755 r(log = log y)	3496				

1/ % Investigat d Stag I started in Nov 87 but the triglyceride measurements began in January
 68 Because of the pointment system the 118 men can b considered as a random sample of
 those inv stigated

Title: III 31
 Author: f u m t t l t l t r o l v a l and a r u a t i g l y c e r i d e v a l u e i n m e n
 Date of mo 40 A₂ 40 42 44 y r No p o n s 75 u₁

St E I 1967 68 Men

Tot hol	Trigly rid valu in mg/100ml (mg%)															
	Class midpoint minus 0.5 mg % (Class width 20 mg %)															
CL midp	20	40	60	80	100	120	140	160	180	200	220	240	260	280	100	20
150 S		1		1	1		2	1	1			1				
170 S		4	4	3	4	4	2	1	1							
190 S	3	1	7	10	6	4	2	2	1							
210 S		7	9	13	17	6	3	3	2							
230 S	4	9	16	19	11	12	6	4	4		1					
250 S		3	15	15	11	7	5	5	2							
270 S		5	20	11	9	7	3	1	3						1	3
290 S	1	4	11	3	3	4	1	3								
310 S			3	2	3	1	1									
330 S		1	2	2	3	1	1			1						
350 S			1	1	1			1								
370 S																
390 S				1												
410 S																
430 S																
450 S																
Total	hol	at	rol	value	(%)	Correlation coefficient				Trilyceride values (y)						
						r(x,y)				Mean y				1cr 20		
						r(log x log y)				Mean log y				4 495		
														S d log y		
														S d		
														4760		

Triglyceride values (y)

Correlation coefficient

Total hol at rol value (x)

n x 251 A₃ d x 42 956 r(x y) 2552 Mean y 107 20 S d y 50 95
 n log x 5 515 S d log = 1619 r(log x log y) 2720 Mean log y 4 495 S d log y 4760

1/ 1 inv tigated tigo I tarted in Nov 67 but the triglyceride measurements began in January
 68 Because of th ppointment system the 407 m n can be consid red as a random sample of
 th s investigated

Table A III 30

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Frequency table of serum total cholesterol values and serum triglyceride values in men
 Number of men 121 Age 37 years Response 71 3 1/

Tot chole		Triglyceride values in mg/100 ml (mg%)															
mg %		Class midpoint minus 0.5 mg %															
		20	40	60	80	100	120	140	160	180	200	(Class width 20 mg %)					
Cl midp													20	240	280	300	≥320
<150	5																
170	5		3	1	1												
190	5		4	4	2												
210	5		2	4	3												
230	5		3	3	3												
250	5		2	5	8			3		1	1						
270	5					1	1										
290	5			2	5	6		5									
310	5		2	2	3	2	6	1	1	1							
330	5		1		4	2		2	4					1			
350	5				1	1	1	1								1	
370	5																
390	5																
410	5										1						
430	5																
450	5																

Total chol sterol values (x)			Correlation coefficient			Triglyceride values (y)		
mean x	268.75	sd x	39.577	r(x, y)	3223	mean y	97.73	sd y
mean log x	5.504	sd log x	1576	r(log x, log y)	4167	mean log y	4.465	sd log y

1/ 1/ investigated stage I started in Nov 67 but the triglyceride measurements began in January 68. Because of the appointment system the 121 men can be considered as a random sample of those investigated.

68 Han

Case I 1967

unusual opportunity

$$y_k^j \quad y_k^i \quad y_k^d$$

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Table

Treatment	Yield (%)	Conc. (%)	Time (min)	Temp. (°C)	pH	Color	Notes
Control	90	10	15	100	7.0	White	
NaOH	85	15	15	100	10.0	Yellow	
HCl	80	20	15	100	4.0	Brown	
K ₂ S ₂ O ₈	75	25	15	100	5.0	Dark Brown	
Catalase	65	30	15	100	6.0	Black	
Peroxidase	60	35	15	100	6.5	Black	
Laccase	55	40	15	100	7.0	Black	
MnO ₂	50	45	15	100	7.5	Black	
FeSO ₄	45	50	15	100	8.0	Black	
Ascorbic acid	40	55	15	100	8.5	Black	
Vitamin C	35	60	15	100	9.0	Black	
EDTA	30	65	15	100	9.5	Black	
DTPA	25	70	15	100	10.0	Black	
NH ₄ NO ₃	20	75	15	100	10.5	Black	
(NH ₄) ₂ CO ₃	15	80	15	100	11.0	Black	
Na ₂ CO ₃	10	85	15	100	11.5	Black	
Na ₂ PO ₄	5	90	15	100	12.0	Black	

R. Don

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Третье и последнее

[illegible]

1/ Investigated Stage I started in Nov 67 but the triglyceride measurements began in January 68. Because of the appointment system the 407 men can be considered as a random sample of those investigated.

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Frequency table of serum total cholesterol values and serum triglyceride values in men
 Number of men 121 Age 37 years Response 71 3 1/

Tot cholo mg %	Triglyceride values in mg/100 ml (mg%)															
	Class midpoint minus 0.5 mg % (Class width 20 mg %)															
	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	≥320
150.5																
170.5		3	1	1												
190.5		4	4	2												
210.5	1	2	4	3	1	1										
230.5		3	3	8	5	4	3	1	1							
250.5		2	5	2	6	6	5									
270.5			2	5	2	6	1	1	1					1		
290.5		2	2	4	2	2	2	4								
310.5		1		1	1	1	1									1
330.5							1									
350.5																
370.5																
390.5										1						
410.5																
430.5																
450.5																

Total chol sterol values (x)				Correlation coefficient		Triglyceride values (y)			
mean x	248.75	S d x		39	577	Mean y	97.73	S d y	50.72
mean log x	5.404	S d log x			1576	Mean log y	4.465	S d log y	4845

1/ Investigated stage I started in Nov 67 but the triglyceride measurements began in January 68. Because of the appointment system the 121 men can be considered as a random sample of those investigated.

T b A III 33 H lth r y the Reykj v k area
 Freq ncy t bl of serum total hol et rol v l s and s rum trigly eride values in men
 Numbe of m n 392 Ag 50 51 52 54 years Re pon 75 74 1/

Tot hol		Trigly erid valu s in mg/100 ml (mg%)															
mg %		Class midpoint minus 0.5 mg % (Class width 20 mg %)															
Cl midp		120	40	60	80	100	120	140	160	180	200	220	240	260	280	300	320
150 5										1							
170 5			1	2	3	5	1	1									
190 5			9	5	7	5	3	2									
210 5	1	6	10	7	5	6	6	5									
230 5		8	18	13	7	6	9	3	1	1	3	1					
250 5	2	8	21	23	17	7	7	4	5	2	2						
270 5	1	4	13	13	17	17	5	4	3	2	1	2					
290 5		1	11	17	17	13	1	2	2	1	3	1	2				
310 5			4	4	7	5	1	2									
330 5		1	6	5	5	4		1									
350 5				2	2	1	1	1	1		1						
370 5																	
390 5						1			1								
410 5					1												
430 5																	
450 5																	

Total chol at rol values (x) Correlation coefficient Triglyceride values (y)

Mean x 260 20 S d x 41 798 r(x y) 2319 Mean y 1 66 27 S d y 1 51 98
 Mean log x: 5 5495 d log = 3613 r(log x log y) 2666 Mean log y: 4 453 S d log y: 4665

1/ % inv stiguated tge I started in Nov 67 but the triglyceride measurements began in January
 68 Because of th appointment system the 392 men can be considered as random sample of those investigated

Table A III 32

Health survey in the Reykjavik area Stage I 1967 - 68 - Men

Frequency table of serum total cholesterol values and serum triglyceride values in men
Number of men 507 Age 46 47 48 49 years Response 77 3%^{1/}

Tot chole.		Triglyceride values in mg/100 ml (mg%)															
mg %		Class midpoint minus 0.5 mg % (Class width 20 mg %)															
Cl midp		≤20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	≥320
≤150 %				1													
170 %			1	3	2	2	1										
190 %			5	9	6	1	1			1							
210 %		2	15	15	16	5	3			2							
230 %			6	23	21	8	8			1							
250 %			11	25	21	20	6			5		2	1				
270 %		1	8	18	20	14	12	6	6	3	2	1	1	1			
290 %			5	7	17	13	7	4	1	4	1	5	2	1			
310 %			2	6	10	8	5	2	1	1	1	3	1	1			2
330 %			3	2	2	2											
350 %			1	2	1	1	1	1	4	1	1	1	1	1	1		
370 %						1					1						
390 %					2	1											
410 %						1	1	1	1						1		
430 %										1							
450 %		1															
Total chol sterol values (x)		Correlation coefficient															
Mean x	261 13 S d x	Triglyceride values (y)															
Mean log x	5 551 S d log x	45 520	r(x y)										2872	Mean y	98 59	S d y	51 28
		1684	r(log x log y)										2988	Mean log y	4 470	S d log y	4847
1/	investigated	Stage I started in Nov 67 but the triglyceride measurements began in January															
	58	Because of the appointment system the 507 men can be considered as a random sample of those investigated															

1/ % investigated Stage I started in Nov 67 but the triglyceride measurements began in January 68. Because of the appointment system the 507 men can be considered as a random sample of those investigated

T bl A III 35 He lth ur ey i the R ykjavik area and serum triglycerid value in men
 Freq n y t bl of rum t tel hol t rol al and serum triglycerid value in men
 Numbe f m 71 Ag 61 y ar Respon 59 61/

Trigly erid val u s in mg/100 ml (mg%)

T t chol (Class width 20 mg %)

mg % Cla s midpoint minus 0.5 mg % (Class width 20 mg %)

Cl midp 120 140 160 180 200 220 240 260 280 300 x 20

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

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1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

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1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

1 3 2 1 1 2 1 1 1 1

Triglyceride values (y)

Mean y 92 52 S d y 44 31

Mean log y 4 426 S d log y 4489

Mean log y 4 426 S d log y 4489

Mean log y 4 426 S d log y 4489

Mean log y 4 426 S d log y 4489

Mean log y 4 426 S d log y 4489

Mean log y 4 426 S d log y 4489

Mean log y 4 426 S d log y 4489

Mean log y 4 426 S d log y 4489

Mean log y 4 426 S d log y 4489

1/ % inv etigated St g I start d in Nov 67 but the triglym rid measurements began in January
 68 Be ause of th appointment system the 71 men can be considered as a random sampl of
 those investig ted

Table A IIII34

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Frequency table of serum total cholesterol values and serum triglyceride values in men
 Number of men 167 Age 56 58 years Response 74 3^{1/}

Tot chole mg %	Triglyceride values in mg/100 ml (mg%)															
	Class midpoint minus 0.5 mg % (Class width 20 mg %)															
	20	40	60	80	100	120	140	160	180	200	220	240	260	280	300	≥320
150.5																
170.5		1		1			1									
190.5		2	2	3			1									
210.5		4	5	3												
230.5		1	10	3	4	6	2	1								
250.5	1	3	8	11	5	2	2	2								
270.5		3	6	11	3	4	4	1	1							1
290.5		1	1	7	5	2	2	1	1	1						
310.5			4	3	1	2	2	1	1							
330.5			3	3	2	1	1	2								
350.5				1	1	2	2	1								
370.5											1					
390.5																
410.5			1													
430.5																
450.5																1

Total cholesterol values (x)			Correlation coefficient		Triglyceride values (y)			
Mean x	258.59	S d x	44	512	Mean y	90.96	S d y	
Mean log x	5.542	S d log x	1727	r(log x, log y)	Mean log y	4.423	S d log y	42.80
1/ ¹ in investigated stage I started in Nov 67 but the triglyceride measurements began in January 68. Because of the appointment system the 167 men can be considered as a random sample of those investigated								

I bl A 11126 Health survey i the Rykja ik area St g I 1967 68 Men
Fr que y t bl of s rum tot l hol t rol valu s and rum triglyc rid valu s in men
Number of me 71 Ag 61 years R sponse 59 63 1/

[illegible]

r430 5		Triglyc ride values (y)	
Correlation coefficient		Triglyc ride values (y)	
Total chol st rol valu s (x)		Mo n y	d y
r(x y)		Mo n y	S d log y
2371	44 257	92 52	44 31
2473	44 257	4 426	4489

1/ I in stigat d Stago I started in Nov 67 but th triglyceride measurements began in Janu ry 68 Becas e of the appointment syst m th 71 man can be consid r d as a random sample of those inve tigated

Table A III 36

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Partial correlation coefficients based on Tables A III 15-35 The
 correlation coefficients of the untransformed data are used
 x = serum β -lipoprotein i = serum total cholesterol j = serum
 triglycerides

Age in years	$r_{ij \ k}$	$r_{ki \ j}$	$r_{jk \ i}$
34	- 0 342	0 768	0 636
37	- 147	582	610
40 42 44	- 210	632	586
46 47 48 49	- 013	571	401
50 52 54	- 050	606	441
56 58	096	306	327
61	- 083	711	340

DISCUSSION

Method

In a health survey such as the present one it is necessary to employ rapid and convenient methods for chemical analysis. For this purpose an AutoAnalyzer (1) was used which in turn affected somewhat the choice of methods.

Beta lipoproteins were quantitated by means of an immunochemical method. This method was described by Heiskell et al [16] and Bergquist et al [17] in 1961.

The advantages of the method are that it is simple and can be rapidly carried out manually and does not require any expensive equipment. However reagents are expensive. The method is specific [16]. No quality control was maintained.

These assays were not started until May 1968 due to initial difficulties among other things an automated method [18] was first tried out but this gave unsatisfactory results.

The advantages of the cholesterol method are that it employs few reagents and is easy to use. Apart from the preparation of reagents and the extraction which was carried out on the day the blood samples were collected the determination was done on the AutoAnalyzer which saved much time and labour. The determination of total cholesterol and triglycerides on the same extract had for greater efficiency. Furthermore the method is sensitive and does not require a large sample.

Block et al [19] have published a comparison of this method and the method of Abell et al [20]. They found all the results of a routine on serum samples from 60 patients to be within 6% of results obtained with the method of Abell et al which is regarded to be both accurate and precise and is frequently used as a reference method.

The method is not very specific since various compounds affect the results. No special precautions were taken because of interfering factors such as vitamin A, bilirubin, polyunsatur-

ated fatty acids and haemolysis [20] [21]

AutoAnalyzer tubing was checked by pinching it between two fingers. As soon as it became stiff or flattened it was replaced. It is not, however, clear how frequently this was done.

The precision of the analyses carried out at the clinic can be gauged from the results of the control serum analyses. As mentioned on page 25 the standard deviation turned out to be 5.7 mg% corresponding to a coefficient of variation < 3%. These results are similar to those of Block et al. [14].

It is difficult to state with confidence the accuracy of the analyses. The results from the control serum (Table A III 5) show that the mean of the values obtained for Hyland Special Serum over the period from January 31st to May 27th was 13.7 mg% lower than the midpoint value for the stated acceptable range ($p < 0.001$) and the mean value obtained for Hyland Normal Serum over the period June 6th to July 4th was 3.4 mg% lower than the midpoint value for the stated acceptable range ($p < 0.05$) and for the period from July 9th to September 24th the difference was 8.7 mg% ($p < 0.001$). Nyegaards Seronorm being used in this period. It should be mentioned that several days or even weeks always elapsed from the time the freeze-dried control serum was dispatched until it was received at the clinic and that the packages were not insulated.

The triglyceride method calls for mainly the following comments in addition to what was said in the discussion of the cholesterol method. The method is a sensitive one. However, several compounds interfere with the assay, chiefly phospholipids and glucose. The mixture of zeolite Ca(OH)_2 and CuSO_4 which was used prevents this interference from phospholipids and from glucose at least up to 250 mg% [13] but higher glucose values are rare.

The procedure used deviated from that of Kassler and Lederer [13] in that delay coils were included in the circuit to prevent an unsteady baseline and badly shaped peaks. Blanks were not run for individual samples.

Analyse were started on January 31st 1968 Control serum was used from January 31st until May 27th 1968 The serum was Hyland Special Clinical Chemistry Control Serum The mean value for 11 assays was 7.9 mg% higher than the midpoint value of the stated acceptable range This difference is significant ($p < .001$)

The precision of the analyses is seen from the results of control serum analyses where the standard deviation was 7.4 mg% ($p = .24$)

The control serum was neither used to evaluate the accuracy of TG measurements nor the accuracy of the cholesterol measurements There is reason to suspect that the long time the control serum spent in transit affected the results

Seasonal variations

Numerous studies have been published on the variability of the serum lipid levels in the individual. Fluctuations during one day and over periods of days, weeks, months and years have been reported. These variations can be considerable depending to some extent on the individual. Although these are of great importance they will only be mentioned occasionally in the following discussion on the presence and the extent of seasonal variations in serum lipids levels.

The survey was being carried out at full rate from the beginning of December until the middle of July or for 7 1/2 months. It is important in relation to the aims of a survey such as this one that the period of the survey covers the greater part of the year. When assessing the health benefits from systematic health surveys it may be unsafe to rely on results from surveys completed in a short time e.g. part of a season. Such short-time surveys have indeed been carried out but can give quite erroneous results since for example an epidemic in progress could have unforeseeable effects on the results. Furthermore such surveys can not yield valuable information on seasonal variations [1]

It is however not sufficient that a survey such as the present one be spread throughout most of the year. It is necessary to ensure that all age groups among the participants are equivalently placed with respect to all conceivable determining factors such as time of year and week and appointment time. As discussed in Report A II it must be concluded that in this respect the survey was successful [1]

Another factor which may however be unimportant at any rate in this country is the selection of age groups. The selection of age groups with say a 5, 10, 15 years etc age difference may be misleading since it is conceivable that an epidemic at birth or at a certain age like for example the Spanish flu in 1918 or a periodic epidemic with e.g. a 10 year period could affect the health of the surviving members of the age group [1]

Ancel Keys et al ([5] p 22) appear to be of a different opinion in 1966. They state: "Each area has its peculiarities that affect practical details of organizing and operating cardiovascular surveys and related field work but the following notes are generally applicable to the studies reported here."

It is difficult to engage first class professional personnel to stay in the field away from their home headquarters for more than a few weeks at a time so the schedules for field work were planned accordingly. Selection of the period for field work in a given area required consideration of the seasonal activity of the subject as well as that of the proposed staff and in general this means concentration of the examinations in a period of not over one month.

This does not constitute a serious difference of opinion since the difficulties mentioned by Keys et al are either non-existent in this country or surmountable at any rate as regards Stage I of this health survey because of a computerized National Roster, a small geographical area and the fact that all the work of the survey was in the hands of local residents.

The earliest reference known to us on the seasonal variation of blood lipid in the Nordic Countries is a paper by Keys, Karvonen and Fidanza (1958) [22]. The subject of the paper is a survey carried out in Finland during 1956-57. The authors mention that in East Finland (Karelia region) the mean values for total cholesterol assays during the months of June 56, September 56 and January 57 increased from just under 200 mg% to just under 300 mg% or by some 100 mg% both in men and women. In West Finland however the corresponding increase is much smaller, some 20-30 mg%. The authors state: "In the east the cholesterol significantly from June to September but in the East this seasonal trend was larger and continued until January when the average was about 100 mg per 100 ml higher than in June. Accordingly in comparing serum-cholesterol levels of population seasonal variation should be considered for the regions such as East Finland where a seasonal difference in climate and mode of life are extreme. It is difficult

for the reader to appraise these statements and the authors do not elaborate these points further

In 1961 J. Paloheimo's Monograph *Seasonal Variations of Serum Lipids in Healthy Men* appeared [23]. It reports the first major study conducted solely for the purpose indicated in the title.

The study was made on a series of 80 healthy men who volunteered for the study viz. 45 policemen (age 25-45 years) employed by the police department of the city of Helsinki (Latitude 60 N) and 35 convicts (age 23-48 years) from the Helsinki Central Prison. Initially the total number was 91 but during the course of the study 11 men had to be excluded owing to illness or accident.

At monthly intervals in the period Feb. 1958 - Jan. 1959 fasting blood samples were collected for lipid analysis. Only the results of serum total cholesterol determinations will be considered here. Cholesterol was determined by the modification of the method of Abell et al. [19] introduced by Anderson and Keys (1956) modified by Konttinen (1959).

The error of measurement was estimated by using independent duplications to be ± 4.0 mg% with 39 degrees of freedom. It is not mentioned if control sera were used.

When testing for seasonal variation the author considered monthly median values rather than monthly mean values and used a model of the type

$$y = \alpha + \lambda \sin \left(\frac{\pi}{6} t + \phi \right) \quad (1)$$

where $t = 1$ (Feb. 1958), $t = 2$ (March), ..., $t = 12$ (January 1959) denotes the average over the period (one year) of the characteristic used (the median). 2λ measure the extent of the seasonal variations since the minimum and maximum value of the function y is $\alpha - \lambda$ and $\alpha + \lambda$ respectively.

The function y can also be written in the equivalent form which was that actually used

$$y = \alpha \quad \rho \sin \frac{\pi}{6} t + \gamma \cos \frac{\pi}{6} t \quad (2)$$

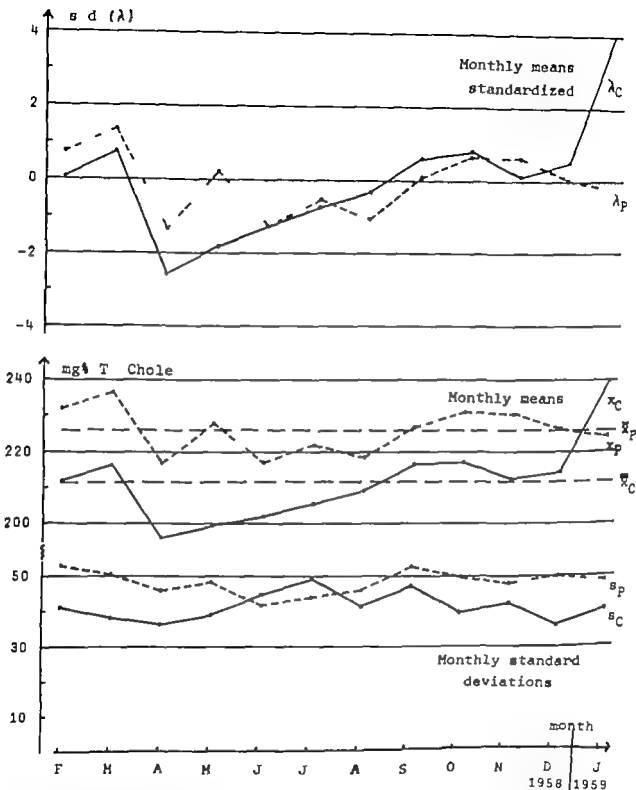
where $\chi^2 = \rho^2 + \gamma^2$ and $\varphi = \arctg(\gamma / \rho)$

Having done more statistical analysis the author concludes Total cholesterol in the group of policemen showed no seasonal variations although the values were lowest in spring and summer. By contrast in the group of convicts total cholesterol fell in summer and rose in winter and the changes were significant ($p < 0.025$). In this group the minimum value for total cholesterol (median) was observed in May (187 mg per 100 ml) the maximum value in January (232 mg per 100 ml). From the values for February and March the drop to the values for April and May was considerable. In the autumn the values began to rise reaching the maximum in the middle of the winter.

At the end of his Discussion the author writes: It appears therefore that it [seasonal variation] is a biological phenomenon the sum of many factors which is not strong enough to be clearly noticeable except under conditions where life is extremely regular as it is in prison and where external influences are slight. Under normal conditions such as were present in the group of policemen the factors influencing the lipid level are so many and so variable that the seasonal trend cannot manifest itself.

The policemen worked by 12 hour shifts but every sixth day as far as from this reason alone it seems doubtful to state that the policemen lived under normal conditions. It should also be mentioned that 11 obs. (2.6%) were missing from the convicts but 48 obs. (8.9%) from the policemen. In the period June-August these numbers were 2 (1.9%) and 28 (20.8%) respectively and in July they were 1 (2.9%) and 15 (13.3%) respectively.

From the curves in Fig. 1 in Palohimo's monograph [29] with periods of 3 month moving averages of median values we see that the extent of the seasonal variation to be about 25 mg% in the group of convicts and about 10 mg% in the group of police.



No of obs pr month Convicts(C) 32 -35 Policemen(P) 30 -44

Figure A III 24 Monthly mean values (\bar{x}) monthly standard deviations (s) and monthly mean values standardized ($\lambda = (\bar{x} - \bar{\bar{x}}) \sqrt{n/s}$)

men When the estimator $2\sqrt{b^2 + c^2}$ is used the estimates become 27.3 mg% and 7.0 mg% respectively

It should be noted that Paloheimo used monthly median values instead of mean values. This has the advantage of being less sensitive for extreme values due to odd mistakes etc.

Fig. A III 24 is analogous to our Fig. A III 14 p. 50 and based on Table 10 in Paloheimo's monograph [23]. There we considered the monthly mean values after exclusion of one policeman with 3 missing measurements. The seasonal variation in the mean values is apparent and seems to be of similar extent as of the median values.

We have done a statistical analysis using model (2). The results were

	Regression Coefficients			Probability	Residual
	a	b	c	p	s.d.
Convicts	211.7	8.31	9.0	0.5	8.8
Policemen	225.8	1.02	4.85	> 1	5.7

Where $\hat{\alpha}$ and $\hat{\beta}$ denote the estimates of the parameters α , β and γ respectively.

The estimator $2\sqrt{b^2 + c^2}$ of the extent of seasonal variation gives 22.1 mg% for the convicts and 9.9 mg% for the policemen. The maximum value of the fitted curves (2) are in December and January respectively.

By comparing the monthly variance in each group with the variance of the monthly mean values about the corresponding empirical curve (fitted curve) it was found that the model fits reasonably well.

(*) Serum total cholesterol values in 25 convicts and 44 policemen
 Helsinki Finland 1958-1959 n = no. of obs. per month
 " serum mean (8 values on Table 10 in [23] J. Paloheimo)

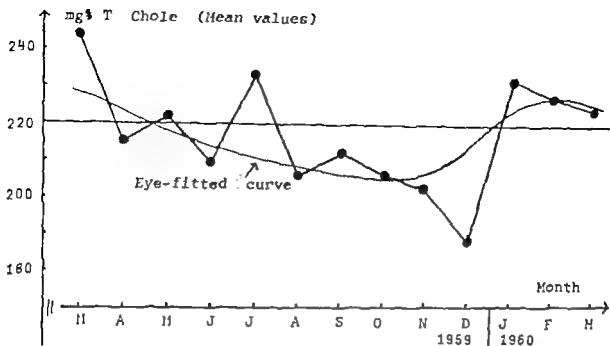


Figure A III 25 From the blood-bank of Bispebjerg Hospital Copenhagen Serum-cholesterol by seasons in 637 blood-donors (men) aged 30-39 years (Based on Fig 3 in [24] Lund Geill and Andresen)

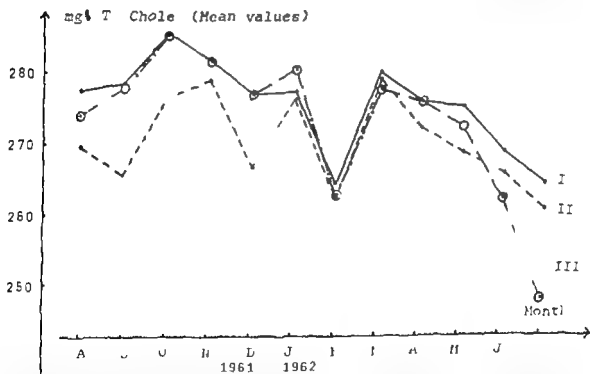


Figure A III b The Stockholm Prospective Study I 1961-62 Serum-cholesterol concentration in healthy men in different months I — age adjusted mean values 1188 men aged 30-64 yrs II --- age adjusted mean values 1265 men aged 15-74 yrs III - - - non-adjusted same 1265 men (Based on Table A 77 in [25] Carlson and Lindstedt)

Since the author did not mention the use of control serum the mean value 280 mg% in January 1958 for the group of convicts must be considered suspiciously high as compared with the second highest value 217 mg% found in Oct 1958. The corresponding median values were 232 mg% and 218 mg% respectively. We have checked that a reasonable lowering of this value will not affect the statistical significance of our test (p = 0.5).

A paper published in 1961 by E. Lund, T. Geili and P. H. Andreassen [24] deals with total-cholesterol measurements on serum from 2399 blood donors at the Bispebjerg Hospital in Copenhagen (Latitude 56 N). 1757 men and 642 women. Blood samples were collected from March 1959 until March 1960 and were all collected in the morning. Donors were not expected to be fasting. These authors state the following on seasonal variations. Fig. 3 shows the seasonal variations in mean cholesterol value for age group 30-39 years. These were exactly the same in the other age groups and there were no distinct fluctuations from month to month. Hansen (1954) and Nergaard (1956) have demonstrated a greater prevalence of coronary thrombosis during the winter months. On the basis of our findings this cannot be a consequence of the serum cholesterol levels being higher during the winter months.

Also in this case it is difficult to appraise the authors' conclusion. They do not mention whether control serum was used or whether the analyses were carried out throughout the period of collection or not. It is not clear whether statistical analysis was done. If the phrase "no distinct fluctuation from month to month"

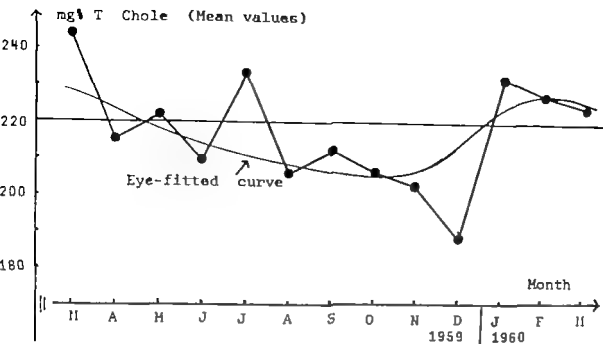


Figure A III 25 From the blood-bank of Bispebjerg Hospital Copenhagen Serum-cholesterol by seasons in 637 blood-donors (men) aged 30-39 years (Based on Fig 3 in [24] Lund Geill and Andresen)

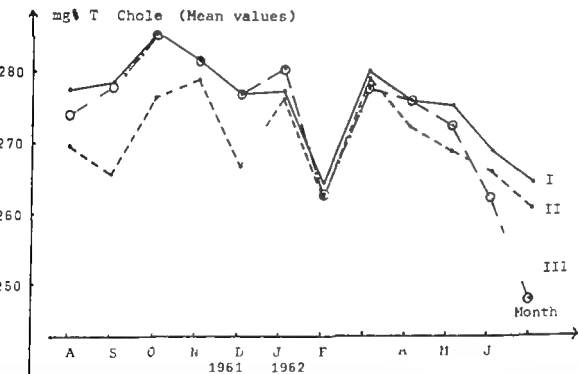


Figure A III 6 The Stockholm Prospective study I 1961 62 Serum-cholesterol concentration in healthy men in different months I — age adjusted mean values 1188 men aged 30-64 yrs II --- age adjusted mean values 1265 men aged 15-74 yrs III - - - non adjusted values 165 men (Based on Table A 77 in Carlson and Lindstedt)

for the distribution of the results of cholesterol assays to be similar to that observed in our survey and assuming that each point represents 50 samples then two standard deviations for each point will be 10 - 13 mg% total cholesterol

If the eye fitted curve is valid then the total cholesterol concentration is at a minimum during September-October and at a maximum during February-March. The extent of this variation will then be some 20 - 30 mg%. Only a statistical analysis of the original data can resolve the question of the validity of these conclusions.

In 1966 L. A. Carlson and S. Lindstedt published the results of analyses including total cholesterol and triglycerides in the serum of some 6500 men and women in Stockholm [26].

Samples were collected at Företagens Hälsokontroll - a health control clinic for the employees of several companies - during the period from August 1st 1961 till July 30th 1962. The authors write the following about monthly mean values of total cholesterol in healthy people (group 10). The values for cholesterol followed the same pattern in men and women during the year (Table A 77 and A 78). Figure 35 shows that the level was fairly constant from January to May but then declined and reached a minimum level in July. The concentration of cholesterol increased from July to September, October and then remained at a rather constant level. The maximal differences in cholesterol level during the year were 38 and 29 mg per 100 ml for men and women respectively.

Carlson and Lindstedt's curve for men is here shown in Fig A III 25 (Curve III 165 men) with the one alteration that the time axis begins in August 1961 when the investigation was started instead of January 1962. Curve III could just as well point to systematic decrease throughout the period as to a seasonal variation. Perusal of Table A 77 reveals however a difference in age distributions for different months. July is peculiarly noticeably different with few and predominantly young men. Therefore curve II of Fig A III 25 is drawn after a correction of the age distribution for each month. This correction

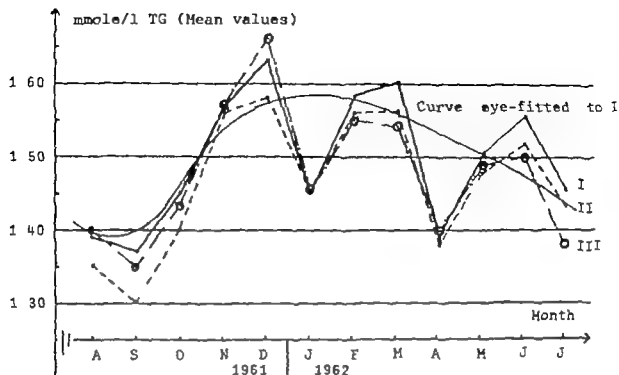


Figure A III 27 The Stockholm Prospective Study I 1961 - 62 Serum triglycerides concentration in healthy men in different months I—age adjusted mean values 1188 men aged 30-64 yrs II---age adjusted mean values 1265 men aged 15-74 yrs III—mean values non-adjusted same 1265 men (Based on Table A 79 in [25] Carlson and Lindstedt)

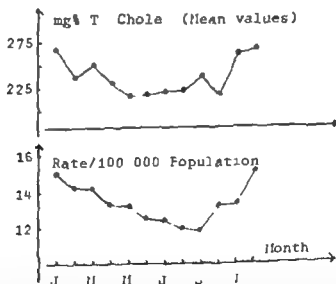


Figure A III 28 Comparison of mean monthly cholesterol levels for 16 prisoners aged 22-28 years with death rates from coronary heart disease in the United States in 1958 In the cholesterol graph the value plotted for December was actually obtained on Dec 4 1956 before the other monthly values It has been transposed to the other end to conform to the calendar year (Based on Fig 3 r 26, Thomas Holljes and Lisenberg)

A graph of average values (Fig 3 Thomas et al) is shown in Fig A III 28 together with a graph of the death rate from coronary heart disease in the USA in 1958. The results of assays for November 1958 were not used in the preparation of the graph. The graph is based on analyses on samples from 16 prisoners. Blood samples were collected after breakfast from 9 00 to 11 00 a.m. There is no mention of a control serum being used. The extent of the variation appears to be about 50 % but it should be noted that the variation for such a small group may deviate markedly from that obtained with a larger sample group.

Finally we shall mention a paper by J T Doyle, E H Kinch and D F Brown which appeared in 1966 [27]. This paper reports a study conducted to determine the presence and extent of seasonal variations in serum total cholesterol concentration in a group of 75 men free of CHD aged 46-62 years. The group was selected at random from the Albany Cardiovascular Health Center, a prospective study of degenerative cardiovascular disease (Latitude 43 N).

Of the 75 men selected, 69 participated throughout the entire period. Of the 69 participants, 52 were seen at monthly intervals from April 1961 to March 1962. The data reported are based on these 52.

Total cholesterol was measured by the method of Abell et al and the analytical procedure was rigorously controlled. The authors state: "All blood samples were drawn after an overnight fast and were assayed in duplicate. The specimen tubes were coded so that all determinations were 'blind'. Duplicate determinations differing by more than 12 mg/100 ml were re-analyzed."

Coded control samples of known cholesterol concentration were routinely included in all series of determinations as a check against a secular trend in the analytical procedure. At the completion of the study, the entire year's samples for some individuals were re-analyzed in a single run. "There were no significant differences in the values obtained in the two procedures."

The authors write about their results: "The mean group level for April was 227 mg/100 ml. The study group mean dropped in the 3 months to a low of 211 mg/100 ml in July. The CFC

being done by weighting the mean cholesterol value in each 5 year cohort in Table A 77" with total number of men in that cohort relative to the grand total number - 1265 Fig A III 26 also shows a curve (Curve I) drawn for the mean values for the age interval 30-64 similarly corrected for age distributions It is hardly justifiable to conclude from curves I and II that a seasonal variation exists

As to seasonal variation in serum triplyceride levels the situation seems to be different Carlson and Lindstedt state

The variation of the triglycerides over the year is given in Figure 36 and Tables A 79 and A 80 The most striking feature was the low level seen in July August and September - for women also in October - which was followed by a pronounced increase to a maximum level in December

Fig A III 27 was drawn after a correction for age like the one carried out for the drawing of Fig A III 26 Two standard deviations for each mean value seem to be about 10 13 mg% From the eye fitted curve the extent of the variation can be estimated to be about 0.2 mmol/l or about 20 mg% The authors do not state whether a control serum was used

Outside the Nordic countries very few investigations into seasonal variations have been carried out In 1961 L B Thomas H W Holljes and F F Eiserberg [25] report the results of monthly measurements of total cholesterol in the serum of 24 healthy white males 20 - 28 years of age all of them prison inmates in Maryland USA (Latitude 39 N) The period of the study was just over a year from the beginning of November 1958 to November 1959 One sample was taken from each inmate each month (with a few exceptions)

The authors make the following statement Highly significant seasonal variations were found with the highest cholesterol values occurring in the winter months and the lowest levels in the late spring summer and early autumn When subjects were grouped according to cholesterol level this cyclic seasonal cholesterol pattern was found to be present in all three groups low intermediate and high

[serum total cholesterol concentration] then rose steadily until November to 233 mg/ 100 ml. In December it dropped to 224 mg/ 100 ml rose in January and February and then began to drop again. The mean body weight remained nearly constant over the entire study period. Data for the 17 additional persons with incomplete observations are similar.

The monthly variation around the mean ranged from 46 mg/ 100 ml in June to 54 mg/ 100 ml in November. The monthly distributions of STCC were similar and differ only in the location of the mean.

Fig A III 29 shows the monthly mean values and the fitted curve (2) (see p 95). The results of the corresponding analysis were

Regression		Coefficients (mg%)		Probability	R residual
a	b	c		p	s d
281.3	6.98	3.98		<0.0005	4.2

Also here we have compared the monthly variance to the variation about the fitted curve and the model (2) seems to fit reasonably well. There seems to be no doubt about seasonal variations and a graphical estimate of the extent is about 17 mg% compared to 16.1 mg% when $2\sqrt{b^2 + \sigma^2}$ is used.

A weakness of the model (2) (or (1)) is the assumption that the time interval of increase is equal to the interval of decrease but when we fit a smooth curve by y_0 (ruling out the possibility of minor peaks [27] [28]) the time interval of increase was always considerably shorter than the time interval of decrease and this also applies to the curves in Fig A III 25-28.

The results from the Icelandic Heart Association's Health Survey do undoubtedly reveal a seasonal variation in serum total cholesterol levels as seen in Fig A III 14 p 50 (compare the standard deviation (3) with those of Fig A III 24 p 96). The extent of the variation during the period December-July appears to be 30 mg% but the yearly variation will probably be somewhat greater. The levels are highest in March but

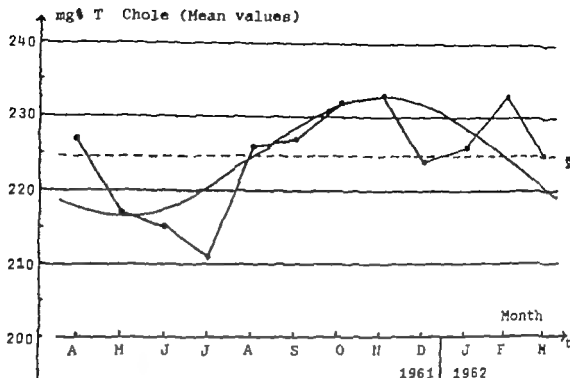


Figure A III 29 From The Albany Cardiovascular Health Center 1961 - 1962 Monthly mean values of serum total cholesterol concentration in 52 healthy men aged 46 - 62 years The least square fitted curve has the equation $Y = 224.7 - 6.98\sin(30t) - 3.98\cos(30t)$ $\bar{X} = 224.7$ mg% = grand mean (Based on Table 1 in [27] J T Doyle et al)

- iv) The period of the survey was rather short and the maximum could fall outside this period
- v) Carlson and Lindstedt did find a variation by some 0 mg% This need not however be in contradiction to our results since the uncertainty of our assays is somewhat larger and the standard deviation for the distribution of triplyceride concentrations is greater than that for the distribution of total cholesterol values See also paragraph iv
- 1) The use of control serum was inadequate (see p 20 26)

As mentioned earlier no control serum was used for beta lipoproteins and the period in which these were measured was short

All this serves to illustrate that knowledge of the seasonal variation of various biological parameters is important for epidemiological studies and that epidemiologists will necessarily have to pay more attention to them than they have done in the past without this knowledge the comparison of different nations is unrealistic

usually the coldest months are January and February. It is interesting to note the absence of any trend (such as a periodic variation) in the weekly standard deviations over this period c f [27]

Arguments supporting the view that the variation is real are

- a) All age groups were equivalently placed throughout the survey period ([1] p 21-25). See also bottom line in Tables A III 6 & 12
- b) A control serum was run during the period from February 1st to July 4th. The results do not suggest any trend neither to decrease nor to increase (Table A III 3 & 4)^{1/}
- c) Various physiological effects due to seasonal variations in environmental factors such as temperature - [29]
- d) The above mentioned investigations - [22] [23] [26] and [27]

For opposing arguments see the section on triglycerides

Analyses for triglycerides were started in January 1968. The results from the Icelandic Heart Association's Health Survey do not suggest any seasonal variation during the period up to the summer vacation which started in early July (Fig A III 19 p 61)

Arguments supporting the view that there is no seasonal variation over this period are the following

- i) See paragraph a) above
- ii) A control serum was run from January 31st until May 27th the results of which do not suggest any systematic variation over this period

The opposing arguments are

- iii) One might expect a variation in triglyceride concentration with the variation of some 30 mg% in cholesterol concentration. On the other hand the correlation coefficient is low ($r \approx 0.25$)

1/ See also appendix I and footnote p 27

- iv) The period of the survey was rather short and the maximum could fall outside this period
- v) Carlson and Lindstedt did find a variation by some 20 mg% This need not however be in contradiction to our results since the uncertainty of our assays is somewhat larger and the standard deviation for the distribution of triglyceride concentrations is greater than that for the distribution of total cholesterol values See also paragraph iv
- vi) The use of control serum was inadequate (see p 20 26)

As mentioned earlier no control serum was used for beta lipoproteins and the period in which these were measured was short

All this serves to illustrate that knowledge of the seasonal variation of various biological parameters is important for epidemiological studies and that epidemiologists will necessarily have to pay more attention to them than they have done in the past. Without this knowledge the comparison of different nations unrealistic

Profiles

For each cohort it seems that the results of analyses for beta-lipoproteins on the one hand and total cholesterol on the other are to a reasonable approximation normally distributed i.e. that conventional models for the statistical analyses of mean values etc. are applicable. On the other hand the distribution of the results for triglycerides seems clearly skew to the right. This is in agreement with the results of other surveys [25] [26]. As seen in the fractile diagrams (Fig. A III 17 p. 59) the results for triglycerides appear to be to an acceptable approximation lognormal in distribution.

The coefficients of variation for the distribution of beta-lipoprotein analyses and cholesterol analyses are low (< 0.3). With coefficients of variation as low as this the logarithm will also be normally distributed to an acceptable approximation [31]. It has not been checked whether the simultaneous distributions for these two compounds or for all three compounds are normal distributions but the interpretation of the correlation coefficients depends partly on whether this is so or not.

Although the results are to an acceptable approximation normally or lognormally distributed one must be cautious to draw on this basis conclusions as to the shape of the tails of the distributions. The tails are especially interesting from the medical point of view, since they mainly provide information on abnormal cases.

The centile-flow diagrams shown in Figs. A III 5, 10 and 18 on pages 32, 44 and 60 were in part prepared to analyze the tails. Curves were drawn through 5%, 10%, 50%, 90% and 95% centiles for the different cohorts in order to reveal how these varied with age. For the same reason the fitted curves on pages 32 b, 44 b and 60 b were prepared for the above mentioned centiles and for the 30%, 70% and 80% centiles too. The fitted curves are drawn by eye and therefore subjective.

Although these measurements are all done in a short period for all the cohorts it is more interesting although more

risky- to consider the graphs as if they had been prepared after observation of one group of men from the age of 34 to the age of 61 that is over a period of 28 years (hence the term flow diagram) It is then possible to interpret the centile flow diagrams as follows

The lower fractiles for the beta-lipoprotein distributions (say below 50%) are virtually independent of age. The highest fractiles (say 90% and higher) seem to be constant up to the age of 50 but then begin to fall off considerably with increasing age. The increase over the last 10 years of the period is some 0.5 mmol/L.

All the fractiles for the cholesterol distributions seem to increase up to the age of 50 but after that the lower fractiles and the median values decrease whereas the higher fractiles show a slight increase. Over the first 15 years the higher fractiles increase by some 20 mg% while the lower fractiles increase by some 10-15 mg%.

The fractiles in the distributions of the triglyceride results are quite interesting. The low and intermediate fractiles show insignificant increases up to the age of 40 and are constant after that age. On the other hand the high fractiles increase considerably up to the age of 50, this increase being some 25 mg% for the 90% fractile and some 50 mg% for the 95% fractile.

Considering the composition of beta lipoproteins one might expect the centile flow diagrams for these to be somewhat intermediate between those for cholesterol and triglycerides. It is interesting that the centile flow diagrams for the latter two compounds in some respect quite dissimilar. This fact together with the low correlation coefficients suggests that high concentration of cholesterol and triglycerides may be somewhat independent risk factors [4].

Decrease after the age of 50 in the higher fractiles in the flow diagrams for the triglycerides could be explained by a higher rate among those with high triglyceride concentrations and/or by changes in food consumption and other habits of life. The trend of the low and intermediate fractiles seems to favour the former explanation. The same might also explain the fact

that the higher fractiles for the cholesterol distributions do not increase after the age of 45

The flow diagrams appear to support the hypothesis that high total cholesterol concentrations are risk factors for men aged 45 - 49 whereas high concentrations of triglycerides are risk factors for men aged 50 - 54 [4] Later stages of this survey may be expected to provide some information on this

It would be interesting to look into the simultaneous effects of these two compounds e.g. by preparing flow diagrams for the sum of their concentrations and or for the sum of the logarithms of their respective concentrations but this will have to be done later

A discussion of how the consideration of flow diagrams leads to ideas on critical values which appear to be in agreement with the ideas of others is given on p 112

Risk factors and normal values

Some of the reasons for considering high blood lipid concentrations to be risk factors for cardiovascular diseases were mentioned in the introduction

The increased risk of coronary heart disease with increased concentrations of cholesterol has chiefly been demonstrated by prospective studies on large groups of people Among the first of such studies was a study of some 2300 men aged 30 - 62 years in Framingham USA which was begun in 1948 and is not yet completed [32] Included in this group were only men who were found free from CHD on entry The average total cholesterol concentration was found to be about 220 mg% the 95% fractile was about 280 mg% and the 5% fractile was about 160 mg%

For young men (30-49 years old) the risk of CHD turned out to be about 11 times higher for those with serum cholesterol of or 280 mg% than for those with less than 220 mg% [32]

In USA it is generally considered that values over 300 mg%

(some say 280 mg%) are abnormal. However American studies indicate that the risk of coronary disease increases with increasing cholesterol concentration - also in the low concentration range [3].

The inhabitants of the Karelia region of Finland have among the highest known values for total cholesterol concentration. They also have the highest known death rate from coronary heart disease (B-26 B-27)^{1/}. In 1961 the death rate was 372 4 per 100 000 for men aged 40-59 years while in the USA the corresponding figure was 365 2 [6].

It is interesting to note that our centile flow diagrams suggest critical values which are in agreement with the figures mentioned above (see Tabl. A III 37 p. 112).

Examination of the centile flow diagrams for cholesterol concentrations on Figs. A III 10 and 10b on pages 44 and 44b reveals that the higher fractiles continue to increase up to the age of 50 but the increase seems to slow down in the age interval 45-49 years and after that it is insignificant. Assuming the main reason for the halt in the increase of the fractiles to be that men with high concentration of blood cholesterol tend to die sooner than others then about 270-280 mg% may be said to be a critical value for men aged 45-49 years in that concentrations above this value are a serious risk factor.

On the other hand the lower fractiles also seem to decrease after the age of 50 which can partly be due to the decrease in the higher fractiles but can also be due to other reasons e.g. changes in food consumption and other living habits. However the words of C. E. Friedberg should be recalled. In all the incidence of subacute coronary heart disease was particularly low when the serum cholesterol was below 200 mg. per 100 ml, suggesting that on a fraction above this value may be abnormal. In most of the studies there was a sharp rise in the subsequent development of coronary heart disease in groups of individuals with serum cholesterol above 240 or 260 mg. per 100 ml. ([3] p. 657).

A similar examination of the fractile flow diagrams for

1/ According to Tabula codificationis minima

Table A.III. 37 Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Approximate range between the 5%- and the 95% centiles in the distribution of A-lipoprotein values
 serum total cholesterol values and serum triglycerides values - and suggested critical levels 1/

Age in years	B-lip in mm Immunoc		T Chole in mg%		TG in mg%	
	5%-95% range	crit level	5%-95% range	crit level	5%-95% range	crit level
35 39	1 4 3 2	2 5	180 - 320	280	40 - 190	140
40 49	1 4 3 1		190 - 330		40 - 200	
50 59	1 4 3 0		190 - 330		40 - 200	

1/ suggested by the flow-diagrams and explained in text and abstract

triglyceride concentrations (Figs A III 18 and 18b p 50) suggests that the critical value is about 140 mg% for men around the age of 50. The low fractile curves are notably horizontal as compared with the highest fractile ones. It is possible that for 2 out of every 3 men the (fasting) triglyceride value is fairly constant while for the third the value is increased for some reason with the result that by the age of 50 it has become a serious risk factor.

Interest in blood triglycerides has been increasing over the last decade. The results of various surveys have suggested that high blood concentrations of these may be a risk factor for coronary heart disease [32].

Albrink [34] is of the opinion that high concentrations of triglycerides are more common in coronary heart disease than high total cholesterol concentrations and that a triglyceride value of 150 mg% is the value which most clearly distinguishes healthy individuals from coronary patients. Some 80% of the coronary disease patients in her study had blood triglyceride concentrations in excess of 150 mg% while only about one third of middle aged men who were considered healthy had these values.

Examination of the centile flow diagrams for beta lipoproteins (Figs A III 5 and 5b p 3 and 32b) reveals that the low fractile curves are virtually horizontal while the high fractile curves begin to fall off considerably after the age of 50. In fact the 95% curve begins to fall off considerably before the age of 50 and the total decrease is about 0.8 mm IC. By arguing in the way as above one may suggest that the critical value is about 2.5 mm IC. It should however be repeated here that the results are few in number and that a quality control was maintained.

In conclusion, the aforementioned critical values attract attention to the curve in Fig A III 21 and 22 on pages 54 and 55. Here there seems to be a change in the upward trend of the graph. The mean values for the beta lipoprotein results have reached about 5 mm IC and the corresponding concentrations for total cholesterol and triglycerides are about 260 mg% and 120 mg% respectively.

Table A III 37 Health survey in the Reykjavik area Stage I 1967 - 68 - Men
 Approximate range between the 5% and the 95% centiles in the distribution of β -lipoprotein values
 serum total cholesterol values and serum triglycerides values - and suggested critical levels 1/

Age in years	B-lip in mm Immunoc		T Chole in mg%		TG in mg%	
	5% 95% range	crit level	5%-95% range	crit level	5%-95% range	crit level
35 39	1 4 3 2	2 5	180 - 320	280	40 - 190	140
40 49	1 4 - 3 1		190 - 330		40 - 200	
50 59	1 4 3 0		190 - 330		40 - 200	

1/ suggested by the flow diagrams and explained in text and abstract

triglyceride concentrations (Figs A III 18 and 18b p 60) suggests that the critical value is about 140 mg% for men around the age of 50. The low fractile curves are notably horizontal as compared with the highest fractile ones. It is possible that for 2 out of every 3 men the (fasting) triglyceride value is fairly constant while for the third the value is increased for some reason with the result that by the age of 60 it has become a serious risk factor.

Interest in blood triglycerides has been increasing over the last decade. The results of various surveys have suggested that high blood concentration of these may be a risk factor for coronary heart disease [34].

Albrink [34] is of the opinion that high concentrations of triglycerides are more common in coronary heart disease than high total cholesterol concentrations and that a triglyceride value of 150 mg% is the value which most clearly distinguishes healthy individuals from coronary patients. Some 80% of the coronary disease patients in her study had blood triglyceride concentrations in excess of 150 mg% while only about one third of middle aged men who were considered healthy had these values.

Examination of the centile flow diagrams for beta lipoproteins (Fig A III d b 32 and 32b) reveals that the low fractile curves are actually horizontal while the higher fractile curves begin to fall off slightly after the age of 50. In fact the 95% curve seems to begin to fall off considerably before the age of 50 and the total decrease is about 1 mm IC. By arguing in the way as above one may suggest that the critical value is about 2.5 mm IC. It should however be repeated here that the results are few in number and that no quality control was maintained.

In connection with the preliminary critical values attention must be drawn to Figs A III 21 and 22 on pages 64 and 65. Here there seems to be a change in the upward trend of the graphs of the mean values for the beta lipoprotein results have reached about 2.6 mm IC and the corresponding concentrations for total cholesterol and triglyceride are about 260 mg% and 120 mg% respectively.

Table A III 38 Medians of the distributions of serum total cholesterol values of male populations in 7 countries^{1/} compared to those of the male population in the Reykjavik area

		Finland		Italy		
		Karelia	West -Finl	Cresvalcore	Montegiorgio	Rome (Railwaymen)
Age						
40	44	285 mg%	248 mg%	194 mg%	192 mg%	207 mg%
45	49	272	255	194 -	192 -	206 -
50	54	262	257 -	198 -	199 -	209 -
55	59	259 -	251 -	204 -	198 -	204 -
Total no of men		814	857	962	710	762
		Netherlands	Greece	U S A (Railroad employees white)		
		Zutphen	Croto	(Switchmen)	(Jedent clerks)(Non-sed clerks)(Executives)	
Age						
40	44	233 mg%	198 mg%	233 mg%	234 mg%	242 mg%
45	49	235	199	232 -	234 -	241 -
50	54	227	210	232 -	232 -	243 -
55	59	226	208	250 -	242 -	247 -
Total no of men		831	652	823	853	247
		Iceland	Japan	Yugoslavia		
		Reykjavik area	Fararumaru	Dalmatia	Slavonia	Velika-Krana
Age						
40	44	246 mg%	167 mg%	182 mg%	196 mg%	154 mg%
45	49	253	165 -	185 -	197 -	157 -
50	54	256	178*	186 -	200 -	159 -
55	59	24	168	188 -	194 -	155 -
Total no of men		817	491	493	666	511

^{1/} 1. 11. 10. 1958 from [B] A. P. P. et al

We have discussed centil flow diagrams at some length. The reason is not that we value highly our speculations at this stage but rather that it seems to us that these diagrams should be useful to epidemiologists and others doing cross sectional studies in the formulation of hypotheses concerning the evolution of most biological values and their potential role as risk factors. There is at any rate no doubt that much information is lost when only means and standard deviations are considered as has been common practice until recently. The only test of the value of the method is experience and there should be room for improvement by using a statistical model which would make it possible to fit the curves more objectively.

The concept of normal values is commonly used. This concept itself and its use have been severely criticized for many years. Therefore normal values will not be further discussed here but for reference a rough estimate is given of the 5% and 95% percentiles for the age groups 35-39, 40-49 and 50-59 years. The interval between these estimates is shown in Table A III. 37 as 5% 95% range but could more conveniently be called 90% common range.

Unfortunately there are no units defined in comparing the results of different laboratories. Different groups of people or different factors can be the groups to be insufficiently compared. Factors are a) different methods used for the selection of the population b) different level of participation c) different analytical methods d) effect of seasonal variations.

In recent years attempts have been made to overcome these difficulties. An example is a coordinated survey carried out in the Netherlands during the year 1957. [6] - Table A II 38

Table A III 39 Serum total cholesterol mean-values (\bar{x}) and standard deviations (s) in apparently healthy men (blood donors) in Norway^{1/} and in North Scotland^{2/}

Location	Age in years	Number (n)	$\bar{x} \pm 2s/\sqrt{n}$ mg%	s mg%	Method
Trondheim Norway	21- 30	24	192 \pm 15	36	Schoenheimer and Sperry (Fasting blood samples)
	31- 40	30	241 \pm 18	47	
	41- 50	25	243 \pm 18	45	
	51- 60	22	272 \pm 27	61	
	61- 70	9	256 \pm 42	59	
North- Scotland	18- 19	12	187 \pm 9	15	Liebermann- Burchard reaction according to the method of Watson (Samples collected during the winter months)
	20- 24	87	214 \pm 6	30	
	25- 29	99	227 \pm 8	40	
	30- 34	77	249 \pm 10	44	
	35- 39	74	261 \pm 10	42	
	40- 44	78	258 \pm 10	45	
	45- 49	69	271 \pm 11	45	
	50- 54	38	253 \pm 15	45	
	55- 59	32	265 \pm 16	44	
	60- 64	21	250 \pm 17	39	
	65- 70	6	243 \pm 32	39	

1/ Based on Table II in [35] (J C Lund et al)

2/ Based on Table IV in [36] (A G Veitch)

page 114 hows the median values of cholesterol results in these countries and in Iceland

On the selection of the population the authors comment as follows The chunk samples dealt with in Europe and Japan though unbiased in respect to the particular areas selected for study are not claimed to be necessarily good samples of the whole region in which they reside let alone of the countries The U railroad employees studied are not claimed to be an ideal sample of all U S railroad employ es; there were too many men in the samples who refus d to cooperate And the Roman sample may be unrepresentative of railroad employees in Italy as a whol But we should not d precate too much the samples are better than thos previously studied ([6] p 15)

The participants w re not required to be fasting when the sample was collected Assays were carried out at laboratories in Minnesota and in Naples where they were sent by post in a dried out form on blotting paper The analyses were done according to the method of Anderson and Keys (1956)

Ex options to this were as stated by the authors Ex options to the practice of making all analyses on dried serum were the Zutphen series in which the analyses of fresh serum were made locally in addition to the analyses of dried serum in M nne ota and at Tanushinaru and Ushibuka where fresh serum after saponification was analysed by the method of colour development with ferric ion intensification (cf latkis et al 1953 H nl y 1957) A few checks made on dried serum samples sent from Japan to Minn sota indicated only fair agreement o the chol st rol values reported here from the Japanese samples may not be perf ctly comparable with the other s ri s ([6] p 11)

Th great variation b tween nations is interesting The hol est rol con entration seems to be greatest among the inhabitants of th Karelia region where the median value for men aged 40 50 y are is about 260 mg% Punning second are the West Finn and th Ic land rs with median values around 250 mg% Further outh the Italians the Yugoslavs and the Greeks have median val about or le s than 200 mg% It should be mentioned

that the Finnish survey was carried out in the autumn and that the participation was more than 97%. Among the Yugoslavs the participation was over 91% and in the rural village of Velika Krana near Belgrade the participation was 96.7% and the median values there were around 150 mg%

Table A III 39 on page 116 shows means and standard deviations for the results of cholesterol measurements in the serum of blood donors in Norway and Northern-Scotland - i.e. in two neighbouring countries where the inhabitants are considered to be related to the Icelanders

The Norwegian survey was done by J C Lund E Sivertsen and H C Godal [35]. Among other things they measured total cholesterol in the serum of 110 clinically healthy men aged 21 - 70 years. Of these 101 were blood donors at a Trondheim blood bank.

The authors make the following statement about the group: "The material presented here cannot with certainty be said to be representative for the population in the City of Trondheim. It consists mainly of blood donors and thus is a pre-selected group. However, self selection within the group has not occurred."

Chemical analyses were done on fasting blood according to the method of Sperry & Webb. The authors do not state whether a control serum was employed nor in which year or at what time of year the samples were collected.

The Scottish study was done by A G Veitch at the Royal Northern Infirmary at Inverness [36]. Total cholesterol was measured in the serum of 93 healthy men aged 18 - 70 years.

The author makes the following comment on the methods:

Samples were collected at the weekly withdrawal sessions during the winter months. Total serum cholesterol was estimated

using the Liebermann-Burchard reaction according to the method of Watson (1960). A commercially prepared control serum was tested with each batch and the standard deviation obtained was ± 8 mg/100 ml.

Comparison of Table A III 39 with the means shown in Table A III 11 on page 43 seems to indicate that the concentration

of cholesterol among Icelandic men is lower than among Scottish men and higher than in Norwegian men. As our results on control serum analyses are low compared with the recommended values as previously mentioned it is possible that the average concentration of cholesterol in Icelandic men is a few mg% higher than our results indicate.

Coordinated surveys of triglycerid concentrations for many different nations have not been reported. Table A III 40 shows the means and standard deviations for the results of triglyceride measurements in the serum of groups of men in Denmark and Sweden.

The Swedish survey is that of Carlsson and Lindstedt [25] which was mentioned earlier. The means in Table A III 40 were calculated from Table A 79 in [25] for the period from February to June 1964.

The Danish survey was done in Ålborg by J. Dyrlberg and E. Hjørne [21].

The author comments as follows on the Ålborg survey: 33 persons aged 11-71 were recruited in the study. Persons with thyroid diseases and coronary atherosclerosis were excluded. Furthermore persons treated with plasma lipid decreasing drugs were excluded and so were persons taking other drugs known to interfere with lipid metabolism such as adrenal steroids and sex hormones.

Overweight was defined as a weight exceeding the ideal weight 5%.

The examination included persons with all degrees of physical activity: laboratory workers, oldiers, clerks, physicians, women, housewives, old age pensioners, and male and female civil defense members. All persons were volunteers and none

Table A III 40 Serum triglycerides mean-values (\bar{x}) and standard deviations (s) in apparently healthy men in Denmark and Sweden

Location	Age in years	Number (n)	$\bar{x} \pm \frac{2s}{\sqrt{n}}$ mg%	s mg%	Method
Aalborg ^{1/} Denmark	11- 20	29	78 \pm 7	20	Eggstein &
	21- 30	24	98 \pm 12	32	
	31- 40	25	102 \pm 19	47	Kreytz (1966)
	41- 50	27	146 \pm 27	70	Fasting blood samples collected March 1969 to Feb 1971
	51- 60	36	117 \pm 18	55	
	61- 70	21	119 \pm 32	72	
Stockholm ^{2/} Sweden	20- 24	6	88		Carlson (1963)
	25- 29	20	109		
	30- 34	39	125		Fasting blood samples collected Feb to June in 1962
	35- 39	89	127		
	40- 44	121	139		
	45- 49	103	147		
	50- 54	93	130		
	55- 59	55	130		
	60- 64	24	122		
	65- 69	10	127		

1/ Based on Table V in [37] (J Dyerberg et al)

2/ Based on Table A 79 in [25] (L A Carlson et al)

were hospital patients 37

Altogether 194 men participated but in Table A III 40 32 of these are excluded due to overweight

Chemical analyses were done on fasting blood using the method of Eggstein and Kreutz (1966) The analytical error for individual analyses was estimated by duplicate measurements as being 6 mg% Control serum was used Due to possible seasonal variations the participants were distributed equally over the period of the survey from March 1969 to February 1971

Comparison of the means obtained in these two surveys with those obtained in the present reveals a much lower level of triglycerides among the Icelanders Even though the samples concerned are not completely comparable this large difference in the mean values (about 10-30 mg%) must be quite unexpected As mentioned earlier the employment of the control serum in the triglyceride assays during this first stage of the survey was inadequate but the results obtained do not suggest that our mean values are too low

In later years a few surveys have been carried out which included measurements of blood beta lipoproteins As we are however not aware of any such survey employing an analytical method which we consider comparable to the one used in this survey no comparison with other surveys is made here

Table A III 40 Serum triglycerides mean-values (\bar{x}) and standard deviations (s) in apparently healthy men in Denmark and Sweden

Location	Age in years	Number (n)	$\bar{x} \pm 2s/\sqrt{n}$ mg%	s mg%	Method
Aalborg ^{1/} Denmark	21- 20	29	78 \pm 7	20	Eggstein E Kreytz (1966) Fasting blood samples collected March 1969 to Feb 1971
	21- 30	24	98 \pm 12	32	
	31- 40	25	102 \pm 19	47	
	41- 50	27	146 \pm 27	70	
	51- 60	36	117 \pm 18	55	
	61- 70	21	119 \pm 32	72	
Stockholm ^{2/} Sweden	20- 24	6	88		Carlson (1963) Fasting blood samples collected Feb to June in 1962
	25- 29	20	109		
	30- 34	39	125		
	35- 39	89	127		
	40- 44	121	139		
	45- 49	103	147		
	50- 54	93	130		
	55- 59	55	130		
	60- 64	24	122		
	65- 69	10	127		

1/ Based on Table V in [37] (J Dyerberg et al)

2/ Based on Table A 79 in [25] (L A Carlson et al)

The fractile flowdiagrams provided an opportunity for a short discussion of blood lipids as risk factors for coronary heart disease in the discussion. In the introduction a few other risk factors were mentioned which will not be discussed here. Coronary diseases are a part of a larger group of diseases termed cardiovascular diseases. Table A III 41 shows the probability for a few different nations that a forty year old man will die of a cardiovascular disease before the age of fifty and sixty. These probabilities are estimated on the basis of figures from 1964 in [34] (S H Preston, H Yeylitz and P Schoen).

Table A III 42 shows the probabilities that a forty year old Icelandic man will die of the most common causes of death before he reaches the ages of fifty, sixty and seventy respectively.

The table shows that cardiovascular diseases are the most common cause of death for Icelandic males in their prime. These probabilities are estimated on the basis of figures from 1964 ([34] Preston et al.).

Table A III 41 Estimated probabilities that a 40 year old man in a specified country will die from cardiovascular disease^{1/} within 10 and 20 years The estimates are based on the 1964 mortality statistics^{2/}

Country	The probability that a 40 year old man will die from cardiovascular disease before reaching the age of	
	Fifty	Sixty
Finland	3 15 %	10 97 %
United States	2 59 -	9 70 -
Scotland	2 44 -	8 41 -
England and Wales	1 77 -	7 16 -
Iceland	1 50 -	5 68 -
Denmark	1 18 %	5 61 %
Germany(Fed Repub)	1 33 -	5 58 -
Italy	1 33 -	5 14 -
Norway	1 00 -	4 78 -
Netherlands	1 22 -	4 76 -
Sweden	0 94 %	4 50 %
Yugoslavia	0 85 -	3 87 -
Greece	0 74 -	2 97 -

1/ Comprises B22 24-29 and A85 - 86 in the Sixth and Seventh Revisions of the International Classification (1948 1955)

2/ The estimates are based on figures in [38] (Preston et al)

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Table A III 42 Estimated probabilities that a 40 year old man will die from specified causes within next 10 20 and 30 years
The estimates are based on the 1964 mortality statistics^{1/}

Causes ^{2/}	Probability that a 40 year old Icelandic man will die before reaching the age of		
	Fifty	Sixty	Seventy
Respiratory T B	0 0 %	0 0 %	0 0 %
B 1			
Other infec and paras.	0 0 -	0 0 -	0 0 -
B 2-17			
Neoplasms	0 5 -	3 7 -	9 5 -
B 18-19			
Cardiovascular	1 5 -	5 7 -	13 7 -
B 22 24-29 A 85-86			
Infl Pneu bronch	0 2 -	0 4 -	0 8 -
B 30-32			
Diarrheal	0 0 -	0 0 -	0 0 -
B 36			
Certain degenerative	0 3 -	0 4 -	1 1
B 20 33 37-38			
Motor vehicle	0 1 -	0 2 -	0 6 -
BE 47			
Other violence	1 25-	2 4 -	3 6
Be 48-50			
Other and unknown	0 4 -	1 0 -	2 9 -
All causes	4 22	13 82 %	32 25 %

1/ The estimates are based on figures in [38] (Preston et al)

2/ According to the International Classification - the Sixth and Seventh Revisions (1948 1955)

ABSTRACT

This report presents the results of a survey of lipoprotein total cholesterol and triglycerides determinations in a male population surveyed 1967-68

This was the first stage (stage I) in a prospective health survey conducted by the Icelandic Heart Association. The area of study was the city of Reykjavik with adjacent suburbs and communes. The population invited was 1/3 of each of 16 year groups of males in the age interval 34-61 years. The population was selected from the National Roster according to birthdays in the 1st, 4th, 7th of each month, the total number being 2,955. The response was 75.1%.

Lipoproteins were determined with the Hyland Beta L Test total cholesterol and triglycerides on Technicon AutoAnalyzer (I) according to Technicon Methodology H-24a and a modification of the method of Kessler and Lederer respectively.

Control sera were run in connection with cholesterol and triglycerides determinations.

Serum cholesterol level was similar to the highest reported from other countries. Contrary to the triglycerides level which are in the low range.

The mean values and lower centiles are practically independent of age whereas the higher centiles are markedly age-dependent.

A seasonal variation for the cholesterol weekly mean values was found. The values were highest in winter and lowest in summer. The difference amounted to about 30-40 mg%. No seasonal variation of triglycerides was found.

Normal flow diagrams for these blood lipids are presented and the use of similar flow diagrams is suggested as an aid to bridge a gap between the interpretation of cross-sectional and longitudinal studies.

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A P P E N D I X

Table A-III-44 Health survey in the Reykjavik area Stage I 1967 - 68 - Men
Deciles of the distributions of serum β -lipoprotein values in men Total response 75 1%^{1/}

Birth year	Age in years of men	No	Deciles ^{2/} in mg%									
			10%	20%	30%	40%	50%	60%	70%	80%	90%	
1907	61	19	18	19	20	20	21	22	22	23	23	
1910	58	16	16	18	20	21	21	25	26	29	38	
17	56	27	18	19	20	21	21	22	23	24	26	
1914	54	33	17	18	20	20	21	24	25	25	28	
16	52	27	18	19	20	20	21	21	24	28	29	
17	51	29	18	19	20	21	23	24	25	30	31	
18	50	27	18	19	20	21	22	24	25	29	31	
1919	49	23	16	17	18	20	21	22	24	26	29	
20	48	52	18	20	21	21	23	26	29	31	31	
21	47	46	16	18	19	20	21	21	24	28	32	
22	46	40	17	20	21	22	23	23	24	26	30	
1924	44	51	18	19	20	21	22	23	24	27	30	
26	42	47	18	19	20	21	22	23	24	25	29	
28	40	48	17	18	20	22	23	23	25	28	31	
1931	37	38	16	18	20	22	23	24	25	30	34	
1934	34	33	18	18	19	20	21	23	25	28	29	

3/ 1/ Investigated The first stage (+)

1/ % investigated The first stage (Stage I) started in Nov 1967 but the β -lipoprotein measurements began in May 1968. Because of the appointment system the 556 men can be considered as a stratified random sample of those investigated.

2/ Cut-off points below which are found 10 20 30 etc percent of the values

Table A III. 55 h 1th survey in th Reykj ik area St I 1987 68 M n Total response 75 1¹/₂

Birth year	Age yrs	No of exam	B-lipoprot ins in mm Immunocrit										b ₁	b ₂	2/ b ₂
			Mean x	s.d. s	s/FN	Min x(1)	Max x(N)	Range x(N)	2nd mom x(1)						
1907	61	63 3	2 04	25	06	1 30	2 40	1 10	08	-1 17	4 96				
1910	58	91 6	2 40	70	17	1 30	3 40	2 50	46	66	3 85				
12	56	61 3	2 15	35	07	1 50	3 30	1 80	12	1 12	5 72				
1914	54	73 3	2 23	46	08	1 40	3 40	2 20	21	74	3 81				
16	52	56 2	2 19	44	08	1 40	3 08	1 60	18	39	2 25				
17	51	69 0	2 37	32	10	1 60	3 60	2 00	26	65	2 68				
18	50	65 8	2 40	76	15	1 70	5 50	3 80	55	2 63	11 36				
1919	49	57 5	2 20	57	12	1 50	4 00	2 50	31	1 44	5 34				
20	48	65 8	2 42	61	08	90	3 40	2 90	36	1 17	2 77				
21	47	65 7	2 27	76	11	80	5 00	4 20	56	1 38	5 67				
22	46	66 6	2 30	60	09	1 50	5 00	3 50	35	2 25	11 67				
1924	44	69 8	2 26	55	08	1 20	4 40	3 20	30	1 21	5 94				
26	42	72 2	2 29	58	08	1 60	4 50	2 90	30	1 88	7 41				
28	40	64 7	2 32	56	08	1 50	4 20	2 70	30	1 09	4 43				
1931	37	67 8	2 39	68	11	1 10	4 20	3 10	46	69	3 32				
1934	34	68 7	2 22	53	09	90	3 40	2 50	28	10	2 97				
Total		556	2 29	58	02	80	5 50	4 70	33	1 35	6 75				

1/ 1/ 1/ investigated (exsained) The first stage (Stage I) started in Nov 1987 but the lipoprotein measurement began in May 1988. Because of the appointment system the 556 men can be considered as stratified random sample of those investigated

2/ m₂ (1 1/N)s₂ b₁ m₃/m₂ b₂ s₄/s₂

1/ % investigated (examined) The first stage (Stage I) started in Nov 1987 but the lipoprotein measurement began in May 1988 Because of the appointment system the 556 men can be considered as a stratified random sample of those investigated

2/ m₂ (1 1/N)s² b₁ m₃/m₂ b₂ m₄/m₂

Table A III ⁴⁵ health survey in the Reykjavik area Stage I 1967 - 68 - Men
Deciles of the distributions of serum total cholesterol values in men Total response 75 1¹/

Birth Year	Age in years	No of men	Deciles 2/ in mg/100ml (mg%)									
			10%	20%	30%	40%	50%	60%	70%	80%	90%	
1937	61	87	196 2	219 3	232 0	238 2	246 6	259 2	281 4	290 1	313 8	
1910	58	85	195 5	224 9	235 5	244 7	253 0	264 5	275 5	291 9	325 5	
12	56	126	202 7	215 1	230 3	246 7	254 5	261 1	273 4	285 8	309 6	
1914	54	127	205 8	219 9	231 9	248 8	256 6	267 9	278 3	295 6	313 8	
16	52	126	194 5	213 6	227 6	240 8	251 1	259 0	269 0	286 1	307 3	
17	51	125	211 2	227 2	237 3	249 4	257 3	265 9	276 4	292 2	308 6	
18	50	117	199 0	221 5	240 6	248 9	257 3	267 0	281 3	291 3	313 5	
1919	49	127	206 7	223 6	236 9	246 6	257 0	268 9	277 8	286 2	303 8	
20	48	175	207 5	222 8	233 9	242 0	250 8	263 0	275 8	287 6	305 5	
21	47	172	203 0	212 8	222 5	234 8	245 5	259 7	271 6	285 2	307 5	
22	46	149	206 6	224 9	238 4	251 2	257 7	269 8	283 6	300 8	316 8	
1924	44	165	197 3	213 8	223 8	233 5	246 4	256 8	268 6	279 8	297 4	
26	42	163	192 6	216 1	226 8	236 3	245 8	259 2	271 6	288 7	310 1	
28	40	160	199 3	214 5	225 2	235 2	245 5	255 2	265 5	280 5	298 5	
1931	37	149	191 8	211 2	223 6	234 0	244 1	255 2	263 0	277 9	294 9	
1934	34	141	190 6	200 0	217 8	231 3	239 1	248 8	258 9	275 8	296 8	

^{1/} investigated

^{2/} Cutting points below which are found 10 20 30 etc per cent of the values

Table A III 47 H lth surv y in the Reykja ík area Stage I 1967 68 M n Total response 76 1%^{1/}
Numerical charcteristic of th distributions of cholesterol v lu in men

Birth year	Ag in years	No of men	Serum total cholesterol in mg%						b_1 b_2	
			Mean \bar{x}	s.d. s	e/YN	Min $x_{(1)}$	Max $x_{(N)}$	Range $x_{(N)} - x_{(1)}$	$x_{(1)}$	$x_{(N)}$
1907	61	87	255 18	45 37	4 86	168 00	380 00	212 00	2034 86	42 2 94
1910	58	85	251 02	44 26	4 80	156 00	355 00	199 00	1936 35	15 2 84
12	56	126	255 78	43 95	3 92	156 00	401 00	245 00	1916 59	56 3 64
1914	54	127	259 28	41 53	3 69	162 00	375 00	193 00	1711 05	29 2 55
16	52	126	249 52	41 30	3 68	143 00	336 00	193 00	1692 47	- 15 2 73
17	51	125	258 92	41 75	3 73	136 00	414 00	278 00	1728 75	27 4 23
18	50	117	260 56	46 13	4 26	172 00	420 00	248 00	2110 01	60 3 92
1919	49	127	258 66	41 03	3 64	172 00	402 00	230 00	1669 86	73 4 50
20	48	175	256 33	45 07	3 41	161 00	512 00	351 00	2019 39	1 40 9 36
21	47	172	252 67	45 84	3 50	166 00	452 00	286 00	2088 98	1 14 5 44
22	46	149	264 44	47 17	3 86	158 00	420 00	262 00	2210 52	1 59 3 54
1924	44	165	248 99	41 65	3 24	169 00	460 00	291 00	1724 39	96 5 92
26	42	163	251 54	46 71	3 64	198 00	450 00	292 00	2168 32	52 4 25
28	40	160	249 04	40 05	3 17	171 00	392 00	221 00	1594 24	67 3 67
1931	37	149	246 19	41 39	3 39	156 00	416 00	260 00	1701 90	72 4 62
1934	34	141	241 99	43 80	3 64	144 00	376 00	234 00	1652 89	47 3 27
Total		2194	253 78	43 83	94	136 00	512 00	376 00	1920 12	66 4 40

1/ % investigated (examined) 2/ m_2 $(1 - 1/N)s^2$; b_1 $m_3/m_2^{3/2}$; $b_2 = m_4/m_2^2$

Table A III 48 Health survey in the Reykjavik area Stage I 1957-68 - Men
Deciles of the distributions of serum triglyceride values in men Total response 75 1% 1/

Birth-year	Age in years	No of men	Deciles 2/ in mg /100ml (mg%)									
			10%	20%	30%	40%	50%	60%	70%	80%	90%	
1907	61	71	47 9	57 4	65 3	72 9	80 0	87 1	105 3	125 0	150 1	
1910	58	70	48 0	56 8	64 2	71 6	78 9	86 3	96 0	108 7	140 5	
12	56	97	52 0	60 9	69 7	77 0	84 2	92 0	104 1	119 7	142 8	
1914	54	105	42 9	53 4	60 9	68 4	79 3	91 9	106 9	123 8	149 1	
16	52	96	45 7	59 0	70 3	77 0	83 6	90 2	105 8	129 9	184 5	
17	51	100	53 5	60 9	68 3	75 5	82 6	89 8	100 5	113 0	145 5	
18	50	91	47 9	59 6	69 7	79 8	89 9	100 6	112 8	141 4	180 0	
1919	49	100	43 0	54 7	65 2	74 7	83 0	91 8	105 2	123 8	165 5	
20	48	140	51 1	59 9	68 6	78 4	88 4	100 1	114 5	140 5	180 5	
21	47	147	43 9	55 4	64 1	72 9	82 1	91 6	107 2	128 3	165 6	
22	46	122	53 6	62 6	71 4	78 6	85 8	94 7	106 9	122 8	162 5	
1924	44	140	45 5	58 6	69 0	80 5	92 1	103 3	116 8	137 2	170 5	
26	42	134	46 8	59 5	70 6	77 7	84 7	93 0	107 1	125 1	166 5	
28	40	135	53 0	62 6	72 2	81 2	90 2	100 5	111 1	128 0	160 5	
1931	37	121	43 6	56 4	67 9	77 2	85 9	97 5	113 3	133 3	154 1	
1934	34	118	37 8	47 7	56 1	64 0	72 2	82 5	93 8	108 5	136 0	

1/ 1/ investigated The first stage started in Nov 67 but the triglyceride measurements began in January 68 Because of the appointment system the 1907 men can be considered as a stratified random sample of those investigated

/ Cutting points below which are found 10 20 30 etc per cent of the values

-_abl A lll 49 l lth y l th Reykj vik re St g I 1987 68 M n
 luro f l cha t i f th d l trib tio s of triglycerides value in men Total respons 75 15 1/

Serum triglycerides in mg%																									
Birth	ye	i	l	M	A	%	Mean \bar{x}	s	d	e/1N	Hl $x_{(1)}$	Hlx $x_{(N)}$	Range $x_{(N)} - x_{(1)}$	2nd mom m_2	b_1	b_2									
1907	61		71		81	6	92	52	44	31	5	26	28	00	238	00	210	00	1935	69	1	31	4	61	
1910	58		70		81	4	88	30	45	72	5	46	33	00	337	00	304	00	2060	01	2	77	14	29	
12	56		97		75	7	92	71	40	71	4	13	30	00	282	00	252	00	1640	47	1	72	7	31	
1914	54		105		82	6	90	90	44	81	4	37	35	00	295	00	260	00	1988	77	1	59	6	70	
16	52		94		75	6	97	94	52	83	5	39	18	00	280	00	262	00	2762	39	1	42	4	75	
17	51		100		80	0	93	13	43	46	4	35	48	00	268	00	220	00	1869	83	1	92	6	82	
18	50		91		77	7	104	13	45	46	6	86	25	00	510	00	465	00	4238	53	3	01	17	76	
1919	49		100		78	7	95	81	55	49	5	55	35	00	355	00	320	00	3048	55	1	94	7	88	
20	48		140		80	0	105	78	61	81	5	22	10	00	450	00	440	00	3792	72	2	15	9	98	
21	47		147		84	9	95	56	50	87	4	20	22	00	281	00	259	00	2569	72	1	38	4	79	
22	46		122		81	3	96	84	42	30	3	83	32	00	230	00	198	00	1774	97	1	22	4	19	
1924	44		140		83	8	100	29	48	22	4	08	13	00	285	00	272	00	2308	46	90			3	90
26	42		134		81	7	97	21	50	20	4	34	15	00	330	00	315	00	2501	28	1	82	7	71	
28	40		135		84	4	103	00	54	36	4	68	30	00	360	00	330	00	2933	27	2	44	11	16	
1931	37		121		81	2	97	73	50	72	4	61	25	00	310	00	285	00	2550	86	1	70	7	42	
1934	34		118		83	7	82	11	42	11	3	88	15	00	320	00	305	00	1757	28	2	03	10	54	
Total			1787		81	1	96	37	50	47	1	19	10	00	510	00	500	00	2546	04	2	01	10	03	

1/	%	Investigated (examined)	2/	m_2	(1	1/N) s^2	b_1	m_3/m_2	b_2	m_4/m_2
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1/ % investigated (examined) 2/ m_2 (1 $1/N$) $^{1/2}$, b_1 $m_3/m_2^{3/2}$, b_2 m_4/m_2^2

Table A III 50

Health survey in the Reykjavik area Stage I 1967 - 68 - Men
Values (x_i) of total cholesterol in pooled serum

Date of extraction	Batch I			
	x_i	$x_{i+1}-x_i$		
1967 8 Dec	254 mg%		Number n	14
12	242	- 12	Mean \bar{x}	249.4 mg%
19	241	- 1	Variance s^2	61.96
21	250	+ 9	s d s	7.87
22	250	0	Min	238
28	238		Max	266
1968 4 Jan	266	+ 28	Range	28
5	248	- 18		
10	248	0		
11	258	+ 10		
16	255	- 3		
17	256	+ 1		
18	246	- 10		
19	240 241 ^{1/}	- 6		

1/ This value is not included in the statistical characteristics

ACCOMPANYING DOCUMENTS

Isopropyl Alcohol



propanol-2, isopropanol, IPA ; IPS.1

Property	Test method	Specified limit
Purity % wt	By difference	98.7 min
Color (Hazen units)	ASTM D1208	5 max
Relative density 20/20°C	SMS 1347†	0.786-0.787
Water % wt	ASTM D1364	0.15 max
Water miscibility	ASTM D1722	Completely miscible
Distillation range at 760 mm Hg	ASTM D1078	
IBP °C		82.0 min
BP °C		83.0 max
Acidity (other than carbon dioxide) as acetic acid % wt	SMS 277	0.002 max
Non-volatile matter g/100 ml	ASTM D1393*	0.002 max

00- water— ketone Ketone (as MEK) determined by SMS 328

Shell test method

1500 ml test sample

This product meets the requirements of BS 983, ASTM D776, DIN 53043, BP and USP

GENERAL INFORMATION

A. Hazard

Highly inflammable. The toxicity is low but continuous inhalation of vapour should be avoided. A with other organic solvents, prolonged exposure may cause irritation to the eyes, mucous membranes and throat.

B. Flash Point

50°F 10°C (IP 170—Abel)

Interpretation

Most few kinds of apparently healthy normal adults show HEMOPROCT values from 1.5 mm to 2.7 mm. Those under 20 usually show values near the low end of this range, while those from the 30 and older age groups tend to be near the top of the range. In general, males are somewhat higher than females. In the same age group, blacks of higher social and pathological serum complex are not likely from Hyland Laboratories.

In spite of individual variations within the various age and sex groups, the HEMOPROCT value is remarkably constant for given individual, unaltered, constant state of health. Once norms have established for an individual, changes due to progress of disease or therapy can be reliably followed.

Comments

- 1 It is not necessary to use fasting specimen for BETA L TEST. For best results, the test should be performed on serum rather than plasma. The serum may be frozen, refrigerated, fresh, or backflowed.
- 2 Use the correct pipet or dropper for each purpose. Hold each vertically when dispensing drops from it. Drop sizes are critical. Behavior tests are performed in facilities control of drops from capillary tubes.
- 3 One of the paper slides furnished recommended on the surface of each test plate does not always seem suitable for use in this test.
- 4 After mixing specimen and patient serum on the slide, as pointed to in the HEMOPROCT Lab in exactly as possible as the method of procedure and layout the tube representative of that on the slide.
- 5 When serum testing is important to hold tube horizontally to keep mixture from moving through slide. Scarcely or best center hole, then give microscopic view.
- 6 Centrifuge spreading lower relative centrifugal force than from mentioned. (Step 5) do not produce uniform packing of the precipitate over with prolonged centrifugation.
- 7 Record before showing evidence of leakage after centrifugation on (apparently shorter) test tubes) and repeat test.
- 8 Occasionally precipitate may pack at top of column where center lodged or may show water level between such precipitates are read and interpreted the usual manner.
- 9 Important to be consistent in technique of performing the test. Gravity of blood testing or use of plastic test position of capillary (inner etc.)
- 10 Rubber balls for capillary tubes are packed inside cap of one tester. To remove balls, take off cap then separate top of cap between thumb and forefinger.

Other Uses — Other Methods

BETA TEST procedure may be used to obtain values for beta lipoprotein concentration by precipitation in lipoprotein. This test using larger amounts and serum and 247 (7) serum then proceeding with conventional cholesterol analysis of the precipitate.

The amounts and structure of cholesterol, the alpha and beta lipoprotein fractions have been obtained by performing micro cholesterol

assays on the supernatant of the mixture of BETA L TEST sediment and patient's serum and the patient's serum alone. The difference, after correction for cholesterol of the BETA L TEST sediment, is the beta lipoprotein cholesterol.

The sediment may be used for determining beta lipoprotein total card blood, urine, or not fluid etc., or in any procedure where immunologically specific precipitation of serum beta lipoprotein is desired.

Use of Drawdown Microhematocrit Centrifuge

The Drawdown Microhematocrit centrifuge uses capillary tubes 33 mm long while the HEMOPROCT tubes supplied with the BETA L TEST are 75 mm in length and are filled to total column height of 80 mm. However, the Drawdown centrifuge may be used with the 33 mm capillaries instead of the longer HEMOPROCT tubes, by substituting the following after Step 3.

- 1 Partially (about two-thirds) fill Drawdown capillary with the mixture of test serum and BETA L TEST sediment, flame seal without scattering, and centrifuge for 5 minutes.
- 2 Markers, or one, the height of the column of precipitate and the height of the total column.
- 3 Calculate the equivalent mm of HEMOPROCT precipitate using the following formula:

$$\frac{\text{height of precipitate column}}{\text{height of total column}} \times 80 \text{ mm}$$

equivalent mm of HEMOPROCT precipitate

The results obtained with Drawdown centrifuge, when calculated as above are directly comparable with those obtained with the later sediment and the Adams instruments except that results obtained with the Drawdown instrument may be slightly less precise because of the shorter, flatter sedimentation shorter column of precipitate with the same degree of procedure or longer one.

Bibliography

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How Supplied

Hyland BETA L TEST contains 100 number 020 020
 Patent No. 2,923,484



BETA-L TEST[®]

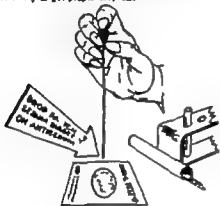
Hyland Antihuman Beta Lipoprotein Precipitin Serum is produced in rabbits by hyperimmunization with human serum beta lipoprotein isolated by ultracentrifugation. This antiserum has been processed to react specifically with beta lipoprotein and to produce rapid and complete precipitation of the low density lipoprotein (density less than 1.063 gm/ml).

Hyland Antihuman Beta Lipoprotein Precipitin Serum is recommended for use in rapid screening test for elevated serum beta lipoprotein levels by the **EMERUCO** method.^{1,2,3}

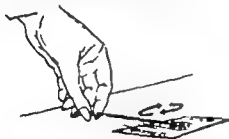
The antiserum contains sodium azide 0.1% as preservative, but care must be taken to avoid contamination. The antiserum should be stored between 2° and 8°C (35° to 46°F) when not in use.

Step 1. Using dropper in bottle, place two drops of Antihuman Beta-Lipoprotein Precipitin Serum on one of the paper BETA L TEST[®] slides provided.

Step 2. Add one drop of patient's serum, using larger (unmarked) capillary tube with bulb attached. Hold capillary tube vertically to get proper drop size.



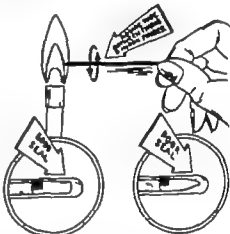
Step 3. Immediately thoroughly mix antiserum and patient's serum with back of finger or applicator held against horizontally one 10 to 12 circular strokes, spreading mixture over the indicated area on the BETA L TEST[®] slide.



Step 4. Holding marked end of EMERUCO[®] tube, quickly lift to bottom of mark. Important—move capillary tube through mixture while allowing tube to fill in order to obtain representative sample. Circular movement is again recommended for consistent results.

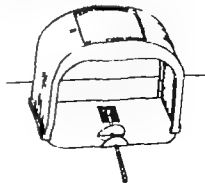


Step 5. Flame-seal end of tube (and seal start). Use point technique as when sealing hemostatic tube. Hold tube horizontally with tip at edge of lower portion of a hot flame to obtain flat glass seal inside. As an alternative to flame sealing, vacuum plastic seals may be used, provided no leakage occurs.



Step 6. Place sealed end of capillary tube toward outside rim of centrifuge and spin for 5 minutes. Use Adams Micro Hemocrit, International Micro Capillary Centrifuge or other types with similar speeds and bead sizes.

Step 7. Read length of column, estimating nearest 0.1 mm. For accurate consistent readings also power, tube holder and millimeter scale provided.



(3) Never use the high pump speed with two-speed pump

(4) During course of run keep reagent sealed in parafilm to minimize moisture pickup from the atmosphere

Operating Sequence:

(1) Pump water through manifold for five minutes prior to operation. During this time check for leaks, pressure build-up, pinched tubing, poor connections, etc.

(2) Following water rinse, lift H reagent lines out of water flowing it to flush through system for three minutes. This prevents subsequent mixing of acid with water which would develop excess in-line and pressure.

(3) Place color reagent line in its bottle and pump color reagent through system. Sample line should be purging water from Sample II wash reservoir. Isopropanol gives slightly higher baseline than water. When reagent baseline is stabilized, set 100% T. The air running through sample line. Also check 0% T and Recorder gain.

(4) Flush sample cups and place on covered tray to inhibit evaporation. Always place two cups of isopropanol before standards to begin run.

(5) At the end of the run, remove the glass assembly line from the color reagent by gently pulling it through and of paper towels. Handle it carefully to avoid splatter.

(6) Allow water to flush through reagent and sample line for three minutes. Then flush water through system for fifteen minutes.

A manifold goes to the pump, the cholesterol color reagent will discolor first becoming milky white then dark brown. This is normal. If tubing shows signs of leaking, stretch and block one notch. Once endblocks have been stretched to the second and third notches do not return to the first notch.

CHOLESTEROL REAGENTS

Cholesterol Color Reagent Anhydrous

Note: All chemicals are J.T. Baker analytical reagents except where other source is specified.

Chemical Composition:

1. Ferric Chloride Anhydrous Reagent, powder a blend (Matheson Coleman & Bell) 825 mg
2. Glacial Acetic Acid conforming III Dichromate Test 2000 ml
3. Conc Sulfuric Acid Reagent Grade 1000 ml

Preparation. In handling and preparing cholesterol color reagent minimize exposure to concentrated acids to atmospheric moisture. Be sure to wear goggles and take necessary precautions in handling these concentrated acids.

1. Place 1600 ml of glacial acetic acid in glass stoppered 2 liter flask. Add the ferric chloride quantitatively with powder from neck of cylinder with 100 ml of glacial acetic acid. Stopper. Place on thermometer and stir with gentle heating until powder dissolves.

2. Allow solution to cool and bring to mark with additional glacial acid.

3. Transfer solution carefully to 4 liter volumetric flask. Add sulfuric acid in portions of 500 ml. Mix by swirling after additions.

4. Seal flask with paraffin foil or parafilm. Allow to cool.

5. Transfer reagent to amber glass bottle. Reagent is stable for at least one month.

Isopropanol

Tech Icon Formal AB-61-60 Reagent Grade Isopropanol

CHOLESTEROL STANDARDS

Stock Standard (5 mg cholesterol per ml)

Chemical Composition:

1. Cholesterol (Eman Organic Chemicals Catalog No. 909) 500 mg.
2. Isopropanol (ACS reagent grade, etc.) 100 ml.

Preparation

1. Place 500 mg cholesterol in 100 ml beaker. Add approximately 50 ml isopropanol.

TECHNICON AutoAnalyzer METHODOLOGY



N method file

N 24a

TOTAL CHOLESTEROL IN SERUM

GENERAL DESCRIPTION

This quantitative procedure for the determination of cholesterol is based on the reaction of concentrated sulfuric acid and ferric chloride in acetic acid with steroids having the 5-one 3 β -ol grouping.

The automation of the colorimetric procedure for cholesterol is described in an article now in press by Block, Jarrett, and Levine. This new methodology is a modification of a previous procedure described by J. Levine and B. Zak (Clin Chim Acta 10 1961 pp 381-84).

A 1 in 20 extract of serum in isopropanol is prepared. An anhydrous color reagent consisting of ferric chloride dissolved in a mixture of acetic and sulfuric acids is used. This reagent is preheated to 95°C in a 40 foot glass coil. After leaving the heating bath the stream is segmented with air and the serum extract is introduced. The reaction takes place in vacuum jacketed mixing coils. Color is developed and read at 550 m μ in a 15 mm tubular flowcell. The method is usable for cholesterol determination ranging from 0 to 400 mg % the basis of 1 in 20 standard.

OPERATING PROCEDURE NOTES

Speed Samples are run at 40 per hour. Precision is slightly improved at 30 per hour.

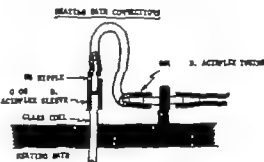
Manifold (see flow diagram)

(1) Standard pump tubes are used for pumping reagent. A Solvaflex sample tube is used for the sample line.

(2) A 12 in. length of 0.081 in. I.D. Acid flex tubing is used to connect the heating bath outlet to the H3 fitting. This length should not be exceeded. Change tubing when it shows signs of swelling.

(3) Supply distilled water to the Sampler II wash well. Water gives a better wash between samples than isopropanol.

Heating Bath Connections: Connect heating bath glass extensions as diagrammed below.



Flowcell May be constructed of standard tubing.

Preparation of Extract: Add 0.5 ml of isopropanol (ACS reagent grade) to a series of test tube via an automatic burette. Pipette 0.5 ml of serum to the isopropanol and cap the tube. Mix immediately to produce a finely divided precipitate. Let stand five minutes then filter or centrifuge taking care not to lose solvent by evaporation. The supernatant extract is then stored in a stoppered tube until ready for use. For analysis fill sample path to the supporting lip otherwise complete sample aspiration may result. When running a plate of samples be sure to use the plastic cover plate to prevent evaporation. Smaller quantities of serum may be used but be sure to maintain the 1 in 20 extract ratio.

Precautions Use of highly concentrated acid color reagent requires several precautions.

- (1) Wear glasses or safety goggles.
- (2) Be sure to have sufficient flow of water flushing out the waste in drains.

(3) Never use the high pump speed with two-speed pump

(4) During course of run keep reagent sealed with parafilm to minimize moisture pickup from the atmosphere

Operating Sequence:

(1) Pump water through manifold for five minutes prior to operation. During this time check for leaks. pins are to be id-up pinched tubing poor connections etc

(2) Flowing water raise 1 ft all reagent lines out of water allowing air to flush through system for three minutes. This prevents base-catalyzed mixing of acid with water which would develop excess heat and pressure

(3) Place color reagent line in 10 ml bottle and pump color reagent through system. Sample line should be aspirating water from Sample II reservoir. Isopropanol flow slightly higher baseline than air. When reagent baseline is stabilized, set 100° T. The water running through sample line also back 0° T and Recorder gain

(4) Fill sample cups and place on covered tray to inhibit evaporation. Always place two cups of isopropanol before standards to begin running from.

(5) At the end of the run remove the glass aspirating line from the color reagent by gently pulling it through wad of paper towl. Handle it carefully it may splatter acid

(6) Allow air to flush through reagent and sample line for three minutes. Then flush water through system for times 1 seven minutes

A manifold gas takes pumping cholesterol color reagent will develop first becoming milky white then dark brown. This is normal. If tubing becomes clogged making stretch-and-blocks one notch. Once endblocks have been stretched to the second and third notches do not return to the first notch.

CHOLESTEROL REAGENTS

Cholesterol Color Reagent Anhydrous

Note: All chemicals are J.T. Baker analytical reagents except where other source is specified

Chemical Composition:

1. Ferric Chloride Anhydrous Reagent, powder, blined (Native on Colman & Bell) 825 mg

2. Glacial Acetic Acid conforming to Dichromate Test 2000 ml

3. Conc Sulfuric Acid Reagent Grade 1000 ml

Preparation: In handling and preparing cholesterol color reagent, minimize exposure to concentrated acid to atmospheric moisture. Be sure to wear goggles and take necessary precautions in handling these concentrated acids

1. Place 1500 ml of glacial acetic acid in glass topped 2 liter flask. Add the ferric chloride quantitatively. Wash powder from neck of cylinder with 100 ml of glacial acetic acid. Stopper. Place on thermometer and stir with gentle heat until powder dissolves

2. Allow solution to cool and bring to mark with additional glacial acid.

3. Transfer solution carefully into 4 liter erlenmeyer flask. Add sulfuric acid in portions of 800 ml. Mix by swirling after additions

4. Seal flask with parafilm or aluminum foil. Allow to cool

5. Transfer reagent to amber glass bottle. Reagent is stable for at least one month

Isopropanol

Technacon Formel AR-41-40 Reagent Grade Isopropanol

CHOLESTEROL STANDARDS

Stock Standard (5 mg cholesterol per ml)

Chemical Composition:

1. Cholesterol (Eastman Organic Chemicals catalog # 309) 500 mg

2. Isopropanol (ACS reagent grade, q.s.) 100 ml.

Preparation

1. Place 500 mg cholesterol in 100 ml beaker. Add approximately 50 ml isopropanol

Mix well and gently heat the isopropanol on a hot plate to dissolve the cholesterol

2 Cool and transfer the isopropanol-cholesterol solution to a 100 ml volumetric flask. Rinse the beaker with isopropanol transfer the rinsings to the flask. Dilute to mark with isopropanol

3 Bottle in 4 oz amber glass bottle with plastic polysac cap

Working Standards

1 Use volumetric glassware for preparation of the dilute standards

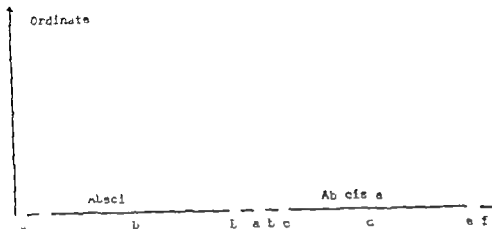
2 Use redistilled isopropanol for preparation of the dilutions

ml of Stock	Dilute to	mg Cholesterol per 100 ml
1	100 ml	5
2	100 ml	10
3	100 ml	15
4	100 ml	20

3 Bottle the dilute standards in 4 oz amber glass bottles with plastic polysac caps

Note: The standards are based upon a 1 in 10 isopropanol extract in the cholesterol determination. They correspond to 100, 200, 300, 400 mg cholesterol per 100 ml. This is multiplied by a dilution factor of 10.

Comments on the histogram



17 at graph is printed out by a computer in virtually
11 printing a column

t 1. After the data is equal to 20 printed
16 is divided into 6 parts as follows (see
for details)

t 1. print a line (1.447)

15 print a line
1 print a line

1

print 15 lines which may have
two lines and represent the tail value
part by 'c' and 'd' and from the order to

t t t t precision in the height of each

Ex The β -lipoprotein values in the interval

$$]0.9 + 0.3(k-1) \quad 0.9 + 0.3k]$$

are presented by the k -th column on the main part D $k=1 \dots 15$
 Values ≤ 0.9 make up the column supported by part B and results
 > 0.9 make up the column supported by F

Comments on the fractile-diagrams

Let $G(x)$ denote the standardized cumulative normal distribution function

Ex 1 Let $H(x)$ denote the (empirical) cumulative distribution function of the β -lipoprotein values

The computer plotted in a coordinate system points (x) with coordinates

$$(1.2 + 0.3k \quad 5 + G^{-1} H(1.2 + 0.3k)) \quad k=0 \dots 14,$$

if $H(1.2 + 0.3k)$ lay in the interval $]0.005 \quad 0.995[$

The values $(1.2 + 0.3k) \quad k=0 \dots 14$ are the right endpoints of the subintervals of part D (see Comments on the histograms)

Ex 2 Let $I(x)$ denote the (empirical) cumulative distribution function of the logarithm of β -lipoprotein values

Ca. $1/15$ of the difference between the logarithms of the endpoints of part D corresponds to the interval size in Ex 1. In this case the width 0.05 was chosen and the points plotted were

$$(0.00 + 0.05k \quad 5 + G^{-1} I(0.00 + 0.05k)) \quad k=0 \dots 14$$

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$k=0 \quad 1$

Acta Medica Scandinavica

Supplementum 617

Management of Hypertension

*Clinical and hemodynamic studies with special reference
to patients refractory to treatment*

By Ove Andersson

Acta Medica Scandinavica

Supplementum 817

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*From the Department of Medicine I
University of Göteborg, Sweden*

Management of Hypertension

*Clinical and hemodynamic studies with special
reference to patients refractory to treatment*

By

Ove Andersson



GÖTEBORG 1977

This summary is based on studies reported in the following papers

- I Prevalence of primary and secondary hypertension studies
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 A study on central and peripheral hemodynamics
 ■ Andersson L Hansson and R Sivertsson
 To be published

- IV Minoxidil in refractory hypertension : Effects on blood
 pressure plasma volume and muscle blood flow
 ■ Andersson and R Sivertsson
 To be published

The papers are referred to in the text by their Roman numerals

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INTRODUCTION

The present study is concerned with the management of hypertension. A model for the management of a large number of hypertensive patients is presented. For this purpose, a description is given of the organization and function of an experimental outpatient hypertension clinic. Special interest has been devoted to problems such as the possibility of reducing blood pressure to an acceptable level in a majority of hypertensive cases derived from screening of a total population sample. Furthermore, important clinical considerations in the treatment of hypertension and side effects of the therapy and patient compliance have been studied.

Another important consideration covered in the present study is the prevalence of secondary curable forms of hypertension in unslected hypertensive patients, as such results should influence the routines for the diagnostic work-up.

Refractory primary hypertension is a condition severe but difficult to handle and dangerous for the patients. It is one of the major concerns in this study. Consequently, hypertensive patients refractory to triple drug treatment have been investigated by means of central and peripheral hemodynamics. They have also been treated in accordance with the findings of these investigations and the results are presented.

RESULTS

PREVALENCE OF HYPERTENSION (Paper I)

PREVALENCE OF SECONDARY HYPERTENSION (Paper I)

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Plasma volume

Regional vascular resistance

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Blood pressure control

Adverse drug effects

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OF REFRACTORY HYPERTENSION (Paper IV)

CONCLUSIONS

ACKNOWLEDGEMENTS

REFERENCES

generally been 70-85%. Such studies have shown that about half of the hypertensive population is unaware of their blood pressure elevation and hence untreated. Of the treated patients a majority has an inadequate blood pressure control. A substantial part has previously been on treatment but has discontinued the medication. Certain studies indicate a drop-out rate from antihypertensive therapy of 50% per year (7-84).

This is the background for the growing interest in procedures aimed at finding and treating all patients with the disease. Furthermore, a new responsibility for the continuous follow-up of the patients is developing. For this reason systems and organizations for better management of hypertension in the community are tested at present (35-71).

Prevalence of secondary hypertension

A review of the literature on secondary forms of hypertension shows a prevalence of about 10% in selected series (18-22, 33). However, the proportion of the total hypertensive population with demonstrable causes of hypertension is not known. It is conceivable that variation due to age, sex, and ethnic and racial differences may exist. It is important to establish the prevalence of secondary hypertension since the diagnostic work-up should largely be aimed at detecting surgically curable forms of hypertension. Thus, the design and scope of the investigations are depending on the expected number of cases with secondary hypertension. Reports from standardized work-up of unselected patient series homogeneous in age and sex are not existing at present.

Refractory hypertension

There is no precise definition of the term 'refractory hypertension'. The general meaning of the term however implies patients resistant to anti-hypertensive therapy.

Poor blood pressure response to single drug treatment associated with sodium retention and expanded extracellular fluid volume has previously been reported (19-23). In these studies blood pressure was well controlled after addition of diuretics. Combined therapy with two or more drugs is also common and many authors point out the advantage of effective blood pressure

PREVIOUS STUDIES

General aspects on the benefit of antihypertensive treatment

Hypertension of primary or secondary origin has been shown to be an important factor in the development of cardiovascular diseases (45 46 77 87) Thus it was reasonable to assume that treatment of hypertension might eliminate or reduce morbidity in hypertensive cardiovascular diseases

The earliest proof of benefit from antihypertensive treatment was the demonstration of an improved prognosis with respect to survival rate in patients with malignant hypertension (57 63 66 69) Shortly thereafter communications from Smirk's group showed an improved prognosis for patients with congestive heart disease or grade II eye-ground changes among treated hypertensive patients (40 70) The prevention of cerebral vascular disease by hypotensive treatment was convincingly demonstrated in several studies (4 41 48) A well controlled study in a selected population of male hypertensives gave evidence of a decrease in the frequency of complications such as congestive heart failure + cerebrovascular accidents and renovascular disease in the treated patients The patients had moderate to severe hypertension with a high frequency of organ damage at entry (79 80)

Further studies are needed to evaluate the benefit from treatment of hypertension in elderly in women and in mild hypertension without hypertensive organ involvement Furthermore no valid proof of primary prevention of myocardial infarction or sudden death by antihypertensive treatment exists at present

A new attitude concerning management of hypertension

Epidemiological studies have generally shown a prevalence of hypertension of 5-20 % (42 76) The participation rate in the screening examinations has

generally been 70-85%. Such studies have shown that about half of the hypertensive population is unaware of their blood pressure elevation and hence untreated. Of the treated patients a majority has an inadequate blood pressure control. A substantial part has previously been on treatment but has discontinued the medication. Certain studies indicate a drop out rate from antihypertensive therapy of 50% per year (7-84).

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Refractory hypertension

The exact clinical definition of the term refractory hypertension. The general meaning of the term however, implies patients resistant to anti-hypertensive therapy.

Poor blood pressure response to single drug treatment associated with sodium retention and expanded extracellular fluid volume has previously been reported (19-23). In these studies blood pressure was well controlled after addition of diuretics. Combined therapy with two or more drugs is also common and many authors point out the advantage of effective blood pressure

sure reduction (37-89). However, also with combined therapy some patients respond inadequately. Reports on the prevalence of this type of refractory hypertension from large treated populations are lacking.

In a definition of refractory hypertension it would be logical to take into account the blood pressure reduction, the blood pressure level achieved and the amount of drugs used. Consequently, in the present study the term 'refractory' has been used in patients on triple drug therapy in doses regarded as optimal in our clinic with blood pressures still above 200/110 mm Hg and a blood pressure reduction of less than 10 %.

Vascular peripheral resistance in arterial hypertension

It is generally accepted that vascular resistance in patients with established primary hypertension is increased while cardiac output is within normal range (27, 28, 51, 64). In early stages, i.e. borderline or labile hypertension, however, cardiac output might be increased as the main hemodynamic characteristic (43). The underlying mechanism by which vascular resistance is elevated is under discussion and several explanations have been proposed.

The role of the sympathetic nervous system for the increased vascular peripheral resistance is not yet quite clear (30). Normal, increased and decreased activity of sympathetic origin by means of urinary or plasma catecholamines has been shown (12). The sympathetic nerve activity has been shown to be increased (11, 50) especially in early phases of hypertension (44) and decreased in later, more severe hypertension (5, 29). Therefore the controversy is probably due to differently selected patient materials.

Another factor of importance for vascular resistance is blood viscosity. It has been reported that patients with primary hypertension have increased blood viscosity (75). Other investigators consider blood viscosity to be within normal limits in essential hypertension. This might simply be a question about methods since in vitro measurements are an unreliable indicator of in vivo viscosity (13).

A plausible explanation of the increased vascular resistance in hypertension is the concept of a morphologic change in the arteriolar wall. Animal

studies from Folkow and his group have demonstrated that vascular flow resistance is increased in hypertensive rats even at maximal flow vasodilation when compared to normotensive rats. This fact strongly suggests reduced vascular lumen in these animals. Furthermore, for any given degree of smooth muscle shortening the curve for blood flow resistance will be steeper in vessels with an increased wall/lumen ratio as compared to normal vessels (25). These observations are also confirmed in man (26-67). This might be one of even the main explanation for the increased 'vascular reactivity' to vasoconstrictor agents often found in hypertensive patients (14-16, 56). The normal threshold of flow resistance increase on infused vasoconstrictor agents in hypertensive patients (67) contradicts the theory of an increased 'vascular reactivity' in the sense of increased arterial smooth muscle sensitivity.

Vasodilating the artery in hypertension

Osmotic drugs apparently reduce arterial pressure by decreasing both total peripheral resistance and arterial fluid and plasma volume (10, 74, 78). Unfortunately, their hypotensive action is rather weak and sufficient only in mild hypertension.

Sympatholytic drugs relax both resistance and capacitance vessels and can depress myocardial function (65). Dox-dependent impairment of sympathetically mediated homeostatic cardiovascular reflexes can cause orthostatic and exercise hypotension and interference by these drugs with other sympathetic functions can also cause gait, intestinal and sexual disturbances.

Direct vasodilator drugs like hydralazine have the advantage of acting on blood vessels and almost exclusively on the arteries while venous capacitance is relatively unaffected (90). However, if not used in combination with adrenergic blocking drugs, hydralazine must be considered relatively ineffective in the treatment of hypertension. Hydralazine alone would not be able to accept reflex activity resulting in increased sympathetic outflow, increased cardiac output and thus limit blood pressure reduction (26, 90). On the other hand, in combination therapy with α -receptor blockade, hydralazine has proved to give effective blood pressure reduction (37, 89). Hydralazine is generally well tolerated in doses of 150-200

mg per day. This dose should however rarely be exceeded because of an increased risk of developing a lupus erythematosus resembling syndrome in patients with low metabolism rate of the drug (58).

Minoxidil; a piperidino pyrimidine derivative is still under clinical investigation. Like hydralazine, this drug acts by direct relaxation of arteriolar smooth muscle (15, 61) and is probably superior to hydralazine in this respect (36). It has sodium retaining properties and should therefore be administered together with diuretic drugs (34, 36). β adrenergic blockers must also be given for the same reason as with hydralazine. Right atrium cardiotoxic lesions in dogs are described and some patients develop different degrees of hypertrichosis (15, 36). In spite of a minor risk of cardiac lesions, never reported in man, minoxidil has gained interest because of potent vasodilating properties necessary especially in severe hypertension.

AIM OF THE PRESENT STUDY

The aim of the present study was to

describe the organization of an out patient hypertension clinic and to present long term results of blood pressure reduction frequency of side-effect and patient adherence in a random population sample of hypertensive middle aged men

establish the prevalence of secondary hypertension in a random sample of middle-aged men in order to get background data for determination of the scope of diagnostic investigations needed in such hypertensive populations

investigate the central and peripheral hemodynamic characteristics of patients with refractory hypertension in order to find a reason for the poor response to the therapy

study the effect on blood pressure plasma volume and muscle blood flow of a potent vasodilating agent (minoxidil) in the treatment of refractory hypertension

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study the effect on blood pressure plasma volume and muscle blood flow of a potent vasodilating agent minoxidil in the treatment of refractory hypertension

PATIENTS

A multifactor primary prevention trial was started in Göteborg in 1970 (85). The intervention group consisted of a randomly selected third of all men aged 47-54 years at entry (born in 1915-1922 or 1924-1925) and living in Göteborg (n=9996). Of the 9996 subjects invited 7455 (75 %) attended the screening examination which included determination of blood pressure, smoking habits and cholesterol. Cut off points for hypertension was systolic blood pressure above 175 mm Hg or diastolic blood pressure above 115 mm Hg at the screening examination and at a re-examination after 1-2 weeks. Patients with current antihypertensive therapy were also invited to follow-up.

Out of 7455 subjects examined 1159 (16 %) had either antihypertensive therapy (n=361) or a blood pressure at the first visit exceeding one or both of the cut off points (n=798). The latter group was invited for a re-examination and 324 (41 %) of them had blood pressures below the limits on this occasion (Table 1). Fifty-eight patients did not attend the re-examination for various reasons. Out of 361 patients who were already receiving antihypertensive treatment 39 had already appointments for follow-up with their physicians. Fifty-two patients either declined further treatment or did not show up for follow-up. Consequently out of 835 patients considered hypertensive 686 (82 %) accepted the invitation and attended the hypertension clinic. Results from these patients are presented in papers I-III.

After three years' treatment 20 patients had blood pressure above 200 mm Hg systolic and 110 mm Hg diastolic on triple drug regimen and were regarded having refractory hypertension. Five of these did not consent to the hemodynamic study planned. Two patients were found to take the drugs irregularly as shown by negative tests for fluorescein in the urine. Two patients had secondary hypertension. The remaining eleven patients were examined with central and peripheral hemodynamics. One examination failed for technical reasons. Results from the remaining 10 patients are presented in papers III-IV.

Table 1 The fate of subjects with blood pressure above 175 and/or 115 mm Hg or current antihypertensive treatment at screening (n 1159)

Untreated (n=798)

SBP \geq 175 and DBP \geq 115 at re-examination	324
Refused to participate	21
Dead or moved	7
Other causes (missed failure to comply etc.)	30
Referred to the hypertension clinic	416

Treated (n=361)

Referred own physician	39
Refused to participate	24
Dead or moved	9
Other causes (missed failure to comply etc.)	19
Referred to the hypertension clinic	270

METHODS

THE SCREENING EXAMINATION

The screening examination in the primary prevention trial was performed in the afternoon between 4 30 and 7 00 p.m. The blood pressure measurement was done by physicians. It was measured after a 4 5 minutes long interview concerning the subjects' physical health and with the subjects sitting. The blood pressure was measured in the right arm. A cuff containing a rubber balloon 12 cm wide and 26 cm long connected to a mercury manometer was used. Diastolic blood pressure was recorded as phase 5 (disappearance of sounds). The measurements were recorded to the nearest 2 mm Hg.

Cut off points for hypertension was blood pressure above 175 (SBP) or 115 (DBP) on two separate occasions within two weeks. These patients but also those already on antihypertensive treatment were referred to the hypertension clinic. Subjects with blood pressure 160/174 (SBP) or 95/114 (DBP) at the first or second examination were invited to annual re-examinations and were subsequently referred to the hypertension clinic if they fulfilled the criteria mentioned.

THE HYPERTENSION CLINIC

Organization

An out-patient clinic for hypertensive patients was started at Sahlgrenska Hospital in 1970. The main objectives were

To treat and follow the hypertensive patients in the primary preventive trial

To be a referral center for patients with hypertension responding inadequately to therapy or having serious side effects during antihypertensive treatment

To constitute a research unit for epidemiological clinical and experimental studies on arterial hypertensive disease

The hypertension clinic is located in the out-patient premises of the Department of Medicine I. The full time personnel consisted of three specially trained nurses and one secretary. All blood pressure measurements, collecting of blood samples as well as part of the history taking were done by the nurses. The secretary had a key role in administrating the follow-up. She also contacted patients who had failed to attend follow-up in order to arrange new appointments. Nine doctors with a special interest in arterial hypertensive disease, one weekly half-day clinic each. The patients generally saw the same doctor or nurse on each visit. The clinic worked almost exclusively on an out-patient basis.

Blood pressures

Routine blood pressures at the hypertension clinic were measured by nurses. The blood pressure was registered in the supine position after 5 minutes rest and after one minute in the standing position. It was measured in the right arm (at the first examination in both arms and the right leg) and recorded to the nearest 2 mm Hg. The diastolic blood pressure was recorded as phase 5, i.e. when the sounds disappeared. A cuff (12 x 26 cm) connected to a mercury manometer was used. The heart rate was measured immediately before the blood pressure measurements in supine position.

Diagnostic examination and follow-up

All patients went through the same examination. The examinations were done stepwise and started with two tests to the nurses for measurement of blood pressure and heart rate usually with a cuff tight between the shoulder. During this time chest X-rays and electrodiagnostic tests were done and sample for blood and urine tests were taken. The blood tests were hemoglobin, sedimentation rate, S-electrolytes, S-creatinine, S-bilirubin, S-alkaline phosphatase, S-ASAT (GOT) and S-ALAT (GPT), S-uric acid, S-cholesterol and S-triglycerides. The urine tests were protein, glucose, sediment and osmolality after 13 hours thirst. If abnormal osmolality was found a desopressin test was done. Isotope renography was carried out in all men born 1915, 1916, 1920 and 1921 (n=287) using the standard

METHODS

THE SCREENING EXAMINATION

The screening examination in the primary prevention trial was performed in the afternoon between 4 30 and 7 00 p.m. The blood pressure measurement was done by physicians. It was measured after a 4-5 minutes long interview concerning the subjects' physical health and with the subjects sitting. The blood pressure was measured in the right arm. A cuff containing a rubber balloon 12 cm wide and 26 cm long connected to a mercury manometer was used. Diastolic blood pressure was recorded as phase 5 (disappearance of sounds). The measurements were recorded to the nearest 2 mm Hg.

Cut off points for hypertension was blood pressure above 175 (SBP) or 115 (DBP) on two separate occasions within two weeks. These patients but also those already on antihypertensive treatment were referred to the hypertension clinic. Subjects with blood pressure 160/174 (SBP) or 95/114 (DBP) at the first or second examination were invited to annual re-examinations and were subsequently referred to the hypertension clinic if they fulfilled the criteria mentioned.

THE HYPERTENSION CLINIC

Organization

An out-patient clinic for hypertensive patients was started at Sahlgrenska Hospital in 1970. The main objectives were

- To treat and follow the hypertensive patients in the primary preventive trial
- To be a referral center for patients with hypertension responding inadequately to therapy or having serious side effects during antihypertensive treatment

To constitute a research unit for epidemiological, clinical and experimental studies on arterial hypertensive disease.

The hypertension clinic is located in the out-patient premises of the Department of Medicine I. The full time personnel consisted of three specially trained nurses and one secretary. All blood pressure measurements, collecting of blood samples as well as part of the history taking were done by the nurses. The secretary had a key role in administering the follow-up. She also contacted patients who had failed to attend follow-up in order to arrange new appointments. Nine doctors with a special interest in arterial hypertensive disease ran one weekly half day clinic each. The patients generally saw the same doctor or nurse on each visit. The clinic worked almost exclusively on an out-patient basis.

Blood pressures

Routine blood pressures at the hypertension clinic were measured by nurses. The blood pressure was registered in the supine position after 5 minutes rest and after one minute in the standing position. It was measured in the right arm (at the first examination in both arms and the right leg) and reduced to the mean ± 2 mm Hg. The diastolic blood pressure was recorded as phase 5, i.e. when the sounds disappeared. A cuff (12-26 cm) connected to a mercury sphygmometer was used. The heart rate was measured immediately before the blood pressure measurements in supine position.

Diagnostic examination and follow-up

All patients went through the same examination. The examinations were done in pairs and started with two visits to the nurses for measurement of blood pressure and heart rate usually with a five minute interval between the readings. During this time chest X-rays and electrocardiograms were done and samples for blood and urine tests were taken. The blood tests were hemoglobin, sedimentation rate, electrolyte, 5-creatinine, 5-bilirubin, 5-alkaline phosphatase, 5-ASAT (GOT) and 5-ALAT (GPT), 5-urate, 5-cholesterol and 5-triglycerides. The urine tests were protein, glucose, sediment and osmolality after 13 hours' thirst. If abnormal osmolality was found a desopresil cannate test was done. Isotope renography was carried out in all men born 1915-1916, 1919 and 1921 (n=287) using the standard

method and apparatus (Nucab Sweden) Intravenous pyelography In most cases rapid sequence pyelography was performed when the history blood or urine samples or the isotope renogram indicated a suspicion about secondary hypertension Thus intravenous pyelography was performed in 52 cases during the initial work up Renal arteriograms to confirm renal vascular disease was performed in 12 cases Urinary catecholamine excretion rate was determined in all 58 men born in 1921 using a method described by von Euler and Floding (20)

In all 20 patients considered refractory to treatment during the third year of follow-up additional diagnostic examinations were carried out in order to identify patients with secondary hypertension Thus renal arteriograms were performed in 15 patients and isotope renograms or rapid sequence pyelograms were done in the remaining 5 patients

At the third visit to the clinic the patients were also examined by a physician who decided whether further diagnostic examinations were needed Drugs were prescribed and plans were outlined for further management Data from this third visit were used for comparison with follow-up data

After the initial phase patients with uncomplicated and adequately controlled hypertension saw the physician once or twice a year Blood pressure controls were done by the nurses at 3-6 monthly intervals For patients with problems of any kind the intervals between controls were adapted individually If needed the patients could get in touch with their physicians via the secretary at the clinic

Data were recorded in a standardized manner for scientific purposes Special data record forms were used for direct coding and computerization

Indications for antihypertensive therapy

Antihypertensive treatment was introduced in

- 1 Patients between 40 and 60 years with repeated blood pressures above 170/105 at rest Under the age of 40 and above the age of 60 the corresponding blood pressure levels have been 160/95 and 180/110 respectively The same blood pressure limits were used for men and women

2 Patients with blood pressures below the above mentioned limits if heredity factors hypertensive organ manifestations or other risk factors for cardiovascular disease implied an increased risk. These decisions have been made at the discretion of the physician.

The aim of the antihypertensive therapy was to reduce the blood pressure to what has been considered normal for the patient i.e. under the age of 40 a blood pressure below 150/90 mm Hg between 40 and 60 years of age below 160/95 and above 60 years below 170/105 mm Hg.

Choice of antihypertensive drugs

The first drug of choice has been a β adrenoceptor blocking agent in doses corresponding to propranolol 80-640 mg/day. Thiazide diuretics and chlorzhalidone have also been commonly preferably used in older patients with severe hypertension where cardiac decompensation was found or anticipated. Diuretics have been used in doses corresponding to 25-75 mg of hydrochlorothiazide per day. A combination of drugs has generally been preferred rather than an excessive increase of the dosage of one single drug. If acceptable blood pressure levels were not achieved with the initially given β adrenergic blocker and/or a diuretic hydralazine (75-200 mg/day) has been added. If the combination of these drugs did not result in adequate blood pressure reduction pironolactone, bethanidine or occasionally high doses of reserpine or minoxidil has been added.

After two years follow-up of the clinic 589/624 (94.4%) patients were on antihypertensive drugs. Of the 32% of the patients received single drug and 68% combined drug therapy. Thus 18% of the patients had diuretics and 14% of the patients had β adrenoceptor blocking drugs. The combination of diuretic and β adrenergic blockers was given to 26% and adrenergic blocker and hydralazine to 24% of the patients. 14% had a triple drug combination consisting of diuretic, β adrenergic blocker and hydralazine while 4% had other drugs or drug combinations.

Registration of adverse drug effects

Withdrawals of drug caused by supposed side-effects were registered at the end of year one and year two. A retrospective analysis of a possible causal relationship between drugs and adverse effects was made by the clinician.

the case records. The judgement of causality was based on laboratory tests, subjective symptoms or signs related in time to the institution of treatment and recovering after withdrawal. Provocation tests were not used. The analysis was done by the author.

INVESTIGATIONS IN REFRACTORY HYPERTENSION

Study plan

Ten of the patients (Group A) refractory to the triple drug treatment with thiazides (hydrochlorothiazide 50 mg daily or bendroflumethiazide 5 mg daily), propranolol 360-480 mg daily and hydralazine 150-225 mg daily were selected and checked for drug defaulting. They were examined in accordance with our routines but renal arteriograms, determination of glomerular filtration rate (GFR) and urine examination of VMA were also performed. None of the refractory patients had a mean arterial pressure reduction of 10 % or more, nor were their blood pressures at the clinic below 200/110 mm Hg.

Another ten patients (Group B) had triple drug therapy and doses as the refractory patients but responded satisfactorily, i.e. they showed mean arterial pressure reductions of 20 % or more and blood pressures at the clinic below 180/100 mm Hg. They were examined according to the same routines and had the same additional diagnostic work-up as the refractory patients. Each patient responding to therapy was selected to match a non-responder for sex, age, weight and renal function as well as untreated blood pressure during their first or second visit to the clinic.

All patients in both groups had primary hypertension and they had eye ground changes corresponding to Grade II of the Keith-Wagener and Barker classification. Further patient data are presented in Table 1.

The patients in both groups were checked for drug defaulting and had 150 µg proterenol infusions to estimate the degree of β -adrenergic blockade. Their acetylation phenotype was determined. Subsequently plasma volume determinations and hemodynamic studies were performed.

Table 1 Age height weight glomerular filtration at (GFR) per treatment auscultatory mean (ml/min/1.73 m²) blood pressure (MAP) heart rate (HR) and heart size (X-ray) refractory patellar (Group A) and patellar corresponding to triple drug therapy (Group B)

Patient	Age (yr)		Height/weight (cm/kg)		GFR (ml/min/1.73 m ²)		Pre-treatment MAP (mm Hg)		Post-treatment HR (beats/min)		Heart size (ml/m ²)	
	A	B	A	B	A	B	A	B	A	B	A	B
AR	54	50	173/105	179/102	89	79	153	156	74	72	610	480
REV	52	53	174/90	175/92	74	84	156	155	80	70	500	435
RES	59	55	172/100	179/99	89	75	153	153	90	92	440	490
LW	52	54	179/90	171/91	8	86	162	160	76	102	590	470
EL	60	59	176/79	175/76	74	72	153	141	97	76	600	520
EE	57	59	174/95	175/94	72	72	160	159	84	86	470	480
BA	57	55	178/83	184/86	80	78	145	147	76	94	470	460
OK	58	55	179/92	169/88	82	76	147	145	56	79	410	470
EA	57	55	177/88	177/86	86	88	155	155	68	72	460	470
MS	50	52	174/98	175/97	79	81	143	145	84	84	600	510
Mean	55.6	54.7	176/92	176/91	81	79	153	152	79	83	515	479
SD	3.37	2.79	2.5/7.8	4.2/7.6	6.1	5.6	6.2	6.6	11.5	10.8	76.8	24.3
Significance	ns		ns		ns		ns		ns		ns	

the case records. The judgement of causality was based on laboratory tests, subjective symptoms or signs related in time to the institution of treatment and recovering after withdrawal. Provocation tests were not used. The analysis was done by the author.

INVESTIGATIONS IN REFRACTORY HYPERTENSION

Study plan

Ten of the patients (Group A) refractory to the triple drug treatment with thiazides (hydrochlorothiazide 50 mg daily or bendroflumethiazide 5 mg daily), propranolol 360-480 mg daily and hydralazine 150-225 mg daily were selected and checked for drug defaulting. They were examined in accordance with our routines, but renal arteriograms, determination of glomerular filtration rate (GFR) and urine examination of VMA were also performed. None of the refractory patients had a mean arterial pressure reduction of 10 % or more, nor were their blood pressures at the clinic below 200/110 mm Hg.

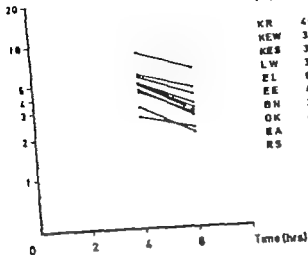
Another ten patients (Group B) had triple drug therapy and doses as the refractory patients but responded satisfactorily, i.e. they showed mean arterial pressure reductions of 20 % or more and blood pressures at the clinic below 180/100 mm Hg. They were examined according to the same routines and had the same additional diagnostic work-up as the refractory patients. Each patient responding to therapy was selected to match a non-responder for sex, age, weight and renal function as well as untreated blood pressure during their first or second visit to the clinic.

All patients in both groups had primary hypertension and they had eye ground changes corresponding to Grade II of the Keith-Wagener and Barker classification. Further patient data are presented in Table II.

The patients in both groups were checked for drug defaulting and had isoproterenol infusions to estimate the degree of β -adrenergic blockade. Their acetylation phenotype was determined. Subsequently, plasma volume determinations and hemodynamic studies were performed.

Patients refractory to treatment

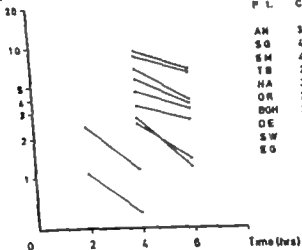
Log plasma isoniazid
conc. $\mu\text{g/ml}$



P.L.	Conc. T1/2
KR	400
KEW	348
KES	310
LW	348
EL	600
EE	418
BH	308
OK	430
EA	448
RS	646

Patients responding to treatment

Log plasma isoniazid
conc. $\mu\text{g/ml}$



P.L.	Conc. T1/2
AM	320
SG	408
SM	425
TB	223
HA	340
OR	228
BOH	215
DE	136
SW	218
EG	423

Fig 1 The result of the Isoniazid acetylation phenotyping of ten patients refractory to triple drug treatment (upper part) and ten patients responding to triple drug treatment (lower part). Patient 1 (EL) is and Isoniazid plasma concentration half-life are labelled to the right. Note that five patients responding to treatment have considerably faster acetylators than any of the patients in the refractory group.

Detection of drug defaulting

All patients defined as refractory to triple drug treatment and occasionally other patients responding inadequately to treatment were given additional placebo treatment with riboflavin (riboflavin 15 mg Beviplex forte^(R) Ferrosan). A qualitative assessment of riboflavin in fresh urine irradiated with ultra violet light by visual observation of a yellow fluorescence was then possible (79)

Determination of acetylation phenotype

The acetylation phenotype in the twenty patients investigated hemodynamically was determined Isoniazid (1-isonicotinyl hydrazine Tibinide^(R) Ferrosan) being metabolized in the same manner as hydralazine (21) was used. The patients fasting ingested Isoniazid 10 mg/kg body weight in the morning. Venous samples were drawn after 2, 4 and 6 hours. The plasma was immediately separated and stored at 20°C until the analysis was performed. The analysis was based on measurements of optical density in a spectrophotometer (53). Plasma concentration half time of Isoniazid was based on the concentrations after 4 and 6 hours in all cases but two where low concentrations allowed only values after 2 and 4 hours.

The results are illustrated in Fig 1. All patients refractory to treatment were shown to be slow acetylators according to plasma half time. In the patients responding to treatment five patients were fast acetylators in two patients (DE, SW) however the concentration of Isoniazid after 2 hours was low and resorption failure or inappropriate dose ingested must be questioned.

Isoproterenol Infusion test

In order to estimate the degree of β adrenergic blockade Isoproterenol in saline solution was infused intravenously (38). In the twenty patients who underwent central and peripheral hemodynamic investigations. With the patients resting comfortably in the supine position and during continuous monitoring of the heart rate with ECG the infusion was started. Initially an Isoproterenol infusion rate of 1 $\mu\text{g}/\text{min}$ was used. After 3 minutes the infusion rate was increased to 3 $\mu\text{g}/\text{min}$ and after another

electrocardiogram from which the heart rate was calculated were recorded on a Mingograph 81 (Siemens Elema). Intra arterial mean pressure (MAP) was obtained from electrically damped curves and measured immediately before each cardiac output determination. Simultaneous direct and indirect brachial artery pressures were determined initially. Auscultatory pressure was recorded in the right arm and diastolic pressure was determined at phase 5. Mean auscultatory arterial pressure (MAP_A) was calculated as the diastolic blood pressure $2/3$ of the pulse pressure.

Cardiac output was determined by the indicator dilution technique using a Cardlognost[®] (Atlas) and Indocyanine Green (Ca di-Green[®]). The blood was reinfused after each determination and the mean from five separate determinations was used. Stroke volume (SV) was calculated by dividing cardiac output (CO) by heart rate (HR). Total peripheral resistance (TPR) was obtained by dividing mean intra arterial blood pressure in the brachial artery (MAP) by cardiac output. It was expressed in arbitrary units (U). CO, TPR and SV were corrected for body surface area (BSA) and the following indices CI , $TPRI$ and SVI were derived.

Regional vascular resistance

Hand blood flow determinations were started two hours after the end of the central hemodynamic investigation. One hour before flow determinations and during the whole experiment the patients were indirectly heated until a temperature of $36.5 \pm 0.2^\circ C$ in order to abolish the sympathetic vasoconstrictor activity of the skin vessels (60 b 62). Hand blood flow ('sympathoplegic flow') was determined by venous occlusion plethysmography and blood pressure recorded in a brachially in the contralateral brachial artery. The water temperature in the plethysmograph was initially kept at $33^\circ C$ and flow recording of blood flow with simultaneous readings of blood pressure were done. The flow resistance during these circumstances was designated 'sympathoplegic resistance' (R_{symp}). The plethysmograph temperature was then increased to $43^\circ C$. After about 3-4 minutes of arterial occlusion during which the patients exercised their hand muscles until ischaemic pain another 5 flow recordings were done. At the highest flow value and the corresponding mean arterial blood pressure intravascular dilution vascular resistance (R_{vi}) was calculated and the mean of the separate determinations of this flow and resistance

3 minutes to 6 $\mu\text{g}/\text{min}$. The heart rate after 3 minutes of the infusion rate at 6 $\mu\text{g}/\text{min}$ was compared to the initial unstimulated heart rate.

A minor but significant increase of the mean heart rate in the twenty patients was noted 2.1 beats/min ($p < 0.001$). The heart rate increase in refractory patients (Group A) was 2.5 beats/min and 1.9 beats/min in patients responding to treatment (Group B) the difference not being significant. No patient had a heart rate increase exceeding 4 beats/min.

Plasma volume determination

Plasma volume was determined in the refractory patients and their matched responding controls. In the ten patients refractory to triple drug treatment plasma volume was also determined after blood pressure reduction on minoxidil treatment.

Plasma volume was determined in the morning after 30 minutes rest in the supine position. The patients had a light breakfast but were not allowed to smoke before the experiments. They had taken their antihypertensive medication immediately prior to the resting period. Evans Blue was injected and blood samples were drawn 5, 10, 15 and 20 minutes after the injection. On the first determination the blood samples were drawn from a central venous catheter but on the re-examination during minoxidil treatment from a peripheral antecubital vein. Plasma concentration of the dye at zero time was estimated from a plot log concentration against time. From that concentration and the originally injected amount of dye plasma volume was calculated (47).

Central hemodynamic studies

The hemodynamic studies were performed in the morning and the patients had taken their antihypertensive medication about one hour earlier.

Polyethylene catheters were inserted percutaneously into the left brachial artery and into a cubital vein. The venous catheter was advanced to the right atrium or centrally in the superior caval vein and the position was checked with X-ray. The intra-arterial blood pressure was recorded with a pressure transducer (Emt 34 Siemens Elema). This pressure and the

electrocardiogram from which the heart rate was calculated were recorded on a Mingograph 81 (Siemens Elema). Intra arterial mean pressure (MAP) was obtained from electrically damped curves and measured immediately before each cardiac output determination. Simultaneous direct and indirect brachial artery pressures were determined initially. Auscultatory pressure was recorded in the right arm and diastolic pressure was determined as phase 5. Mean auscultatory arterial pressure (MAP_A) was calculated as the diastolic blood pressure + 1/3 of the pulse pressure.

Cardiac output was determined by the indicator dilution technique using a Cardiognost[®] (Atlas) and Indocyanine Green (Cardio-Green[®]). The blood was reinfused after each determination and the mean from five separate determinations was used. Stroke volume (SV) was calculated by dividing cardiac output (CO) by heart rate (HR). Total peripheral resistance (TPR) was obtained by dividing mean intra arterial blood pressure in the brachial artery (MAP) by cardiac output. It was expressed in arbitrary units (U). CO, TPR and SV were corrected for body surface area (BSA) and the following indices CI, TPR_I and VI were derived.

Regional vascular resistance

Hand blood flow determinations were started two hours after the end of the central hemodynamic investigation. One hour before flow determinations and during the whole experiment the patients were indolently treated until intense peripheral vasodilation in order to abolish the sympathetic vasoconstrictor activity to the skin vessels (60 b 62). Hand blood flow (sympathoplegic flow) was then determined by venous occlusion plethysmography and blood pressure recorded intra arterially in the contralateral brachial artery. The water temperature of the plethysmograph was initially kept at 33°C and five recordings of blood flow with simultaneous reading of blood pressure were done. The flow resistance during these circumstances was designated sympathoplegic resistance (R_{symp}). The plethysmograph temperature was then increased to 43°C. After about 3-4 minutes of arterial occlusion during which the patients exercised their hand muscles until a clonic pain another 5 flow recordings were done. From the highest flow rate and the corresponding mean arterial blood pressure resistance to maximal dilatation or minimal resistance (R_{min}) was calculated and the mean of these separate determinations of this flow and resistance

3 minutes to 6 $\mu\text{g}/\text{min}$. The heart rate after 3 minutes of the infusion rate at 6 $\mu\text{g}/\text{min}$ was compared to the initial unstimulated heart rate.

A minor but significant increase of the mean heart rate in the twenty patients was noted: 2.1 beats/min ($p < 0.001$). The heart rate increase in refractory patients (Group A) was 2.5 beats/min and 1.9 beats/min in patients responding to treatment (Group B), the difference not being significant. No patient had a heart rate increase exceeding 4 beats/min.

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Plasma volume was determined in the refractory patients and their matched responding controls. In the ten patients refractory to triple drug treatment plasma volume was also determined after blood pressure reduction on minoxidil treatment.

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Central hemodynamic studies

The hemodynamic studies were performed in the morning and the patients had taken their antihypertensive medication about one hour earlier.

Polyethylene catheters were inserted percutaneously into the left brachial artery and into a cubital vein. The venous catheter was advanced to the right atrium or centrally in the superior caval vein and the position was checked with X-ray. The intra-arterial blood pressure was recorded with a pressure transducer (Ert 34, Siemens Elema). This pressure and the

acceptable blood pressure (below 170/105 mm Hg) was achieved

During the first two months the patients were examined every week concerning blood pressure heart rate weight and side-effects. Later these examinations followed every month. S-electrolytes and S-creatinine were followed every month.

Plasma volume determinations and peripheral blood flow examinations of the calf were done when the patients had achieved acceptable blood pressure control. The results were compared with those achieved on the previous therapy.

STATISTICAL METHODS

Standard methods were used for calculation of the mean (\bar{x}) the standard deviation (SD) the linear correlation coefficient (r) and the variation coefficient. The hypothesis of no difference in means was tested with t test for paired observations. In the comparisons between patients refractory to treatment and their matched controls a t test for independent data was used. The hypothesis of no difference in proportions between two groups was tested with the χ^2 squared test. Only two tailed tests were used and differences were considered significant for p -values of 0.05 or less.

was used. The ratio between blood flow resistance at rest and that during maximal vasodilatation will be referred to as sympathoplegic tone as it is regarded to reflect the extent of smooth muscle contraction of the resistance vessels in the hand without any vasoconstrictor nerve influence.

The calf muscle blood flow and resistance determinations were performed the day after the central hemodynamic investigations and in the ten patients refractory to treatment when blood pressure reduction on minoxidil was considered adequate. It was performed in the morning and the patients had taken their antihypertensive medication. Initially the arterial circulation was examined by oscillometry (34). No patient showed evidence of large vessel abnormalities. The calf muscle flow was recorded with venous occlusion plethysmography using the strain gauge technique (82). The blood pressure was measured indirectly in the arm simultaneously with the flow determinations. Initially five readings were made with the patient resting and the resistance at rest was calculated. Then arterial occlusion was applied during about 3-4 minutes and the patients exercised the calf by pedalling on a special foot ergometer until ischemic pain. The pedalling time was measured. After the arterial occlusion was released five recordings of blood flow were made. The highest flow value and the corresponding mean blood pressure was used to calculate resistance at maximal dilatation or minimal resistance (R_{min}). As a rule the mean of three separate determinations was used. The ratio between resistance at rest and the resistance at maximal dilatation will be referred to as vascular resting tone considered reflecting the extent of smooth muscle contraction of the resistance vessels in the calf at rest.

INVESTIGATIONS DURING MINOXIDIL TREATMENT IN REFRACTORY HYPERTENSION

Study plan

When the ten patients refractory to triple drug treatment were found to have increased vascular peripheral resistance it was logical to change the vasodilating therapy. Consequently hydralazine was discontinued and replaced initially with minoxidil 2.5 mg b.i.d. The doses of the other drugs (thiazides and propranolol) were kept constant. Subsequently the minoxidil dose was increased with 5 mg daily at weekly intervals until

to define in functioning tissue. It can be concluded however that ischemic work produces an extremely powerful vasodilatation in the calf muscles at present considered close to maximal and expressed as 'maximal blood flow' throughout the present paper.

To test the variability of the maximal blood flow determinations after ischemic work, repeated measurements in the twenty patients studied were used. The variation coefficient for three consecutive determination of maximal blood flow was 9.1%. A gradual increase of the consecutive maximal flows were observed. Thus the means for the first, second and third determination were 38.5, 39.7 and 41.3 ml/100 ml/min respectively.

Maximal hand blood flow

The method used for dilatation of the hand vascular bed in the present study has previously been discussed by Silfversson (67). Thus it was argued that acetylcholine, histamine or adenosine triphosphate given into the brachial artery could not dilate the hand vessels to the same extent as the method used. It was further observed that large doses of norepinephrine given in the brachial artery were completely overridden by the dilatation procedure.

The variation coefficient for three consecutive determinations of maximal blood flow in twenty-four normotensive patients was 6.9%. No tendency was observed towards increased maximal blood flow with repeated measurements.

MEASUREMENT OF VASCULAR RESISTANCE

Calf

The calf vascular resistance in the calf was based on plethysmographic blood flow determination in the calf and simultaneous measurements of indirect blood pressure in the right femoral artery. During rest in the comparison between the active patient and the passive patient the indirect brachial blood pressure was the constant but significantly increased 112 ± 15.4 mm Hg (SD) as compared to the arterial blood pressure during cardiac output determinations 106.6 ± 16.0 mm Hg (x ± SD). The correlation between these blood pressure measurements was significant ($r = 0.98$, $p < 0.001$).

CRITICAL ASPECTS OF THE METHODS

PROCEDURE TO ESTABLISH MAXIMAL VASODILATATION

Maximal calf blood flow

The accuracy and reproducibility in the establishment of maximal vasodilatation is crucial in the study of structural vascular changes suggested to be the reason for increased peripheral resistance in hypertensive patients (24-67). Folkow showed that heating, arterial occlusion and muscle work could induce a powerful vasodilator stimulus in the forearm almost maximal after about 5 minutes arterial occlusion (24). Later Conway used arterial occlusion to produce hyperemia but also infused adenosinetriphosphate in to the brachial artery during measurements of blood flow. No increase in maximal flow resulting from the infusion was found (9). Furthermore reactive hyperemia has been shown to overcome the effects of intra-venously administered vasoconstrictor drugs (9-24).

Concerning the procedure to produce maximal dilatation of the calf blood vessels it is shown in the present study that 3-4 minutes of arterial occlusion superimposed by muscle work is a powerful vasodilator stimulus (Tables XI-XIV). Considerably large doses of Isoproterenol (3 µg/min) given in the femoral artery after the arterial occlusion was released did not increase the vasodilatation produced by arterial occlusion and muscle work alone in another study (39). However the vasoconstriction induced by large doses of norepinephrine (3 µg/min) was not completely overridden by ischemic work. Preliminary results with acetylcholine (1) indicates that a further increase in vasodilatation after ischemic work might be achieved. However the striking skin hyperemia observed indicates that an increased skin blood flow was added to the muscle blood flow.

Whether a complete smooth muscle relaxation is achieved during maximal vasodilatation is difficult to prove especially as this entity is difficult

to define in functioning tissue. It can be concluded however that ischemic work produces an extremely powerful vasodilatation in the calf muscles at present considered close to maximal and expressed as 'maximal blood flow' throughout the present paper.

To test the variability of the maximal blood flow determinations after ischemic work repeated measurements in the twenty patients studied were used. The correlation coefficient for three consecutive determination of maximal blood flow was 0.91. A gradual increase of the consecutive maximal flows were observed. Thus the means for the first, second and third determination were 38.5, 39.7 and 41.3 ml/100 ml min respectively.

Maximal hand blood flow

The method used for dilatation of the hand is well established in the present study and previously been discussed by Silverstam (67). Thus it was argued that a tylosine, histamine or adenosine (phosphate) given into the brachial artery could not dilate the hand vessels to the same extent as the method used. It was further observed that large doses of norepinephrine given into the brachial artery were completely overridden by the dilatation procedure.

The correlation coefficient for three consecutive determination of maximal blood flow in twenty-four normotensive patients was 0.69. No tendency was observed towards increased maximal blood flow with repeated measurements.

MEASUREMENT OF VASCULAR RESISTANCE

Calf

The calculation of vascular resistance in the calf was based on plethysmographic blood flow determination in the calf and simultaneous measurements of indirect blood pressure in the right arm. During rest in the comparison between healthy patients and responders to therapy the indirect brachial blood pressure was the constant but significantly increased 111 ± 15.4 mm Hg (SD) compared to the arterial blood pressure during cardiac output determination 106.6 ± 16.0 mm Hg (x SD). The correlation between these blood pressure measurements was significant ($r = 0.85$, $p < 0.001$).

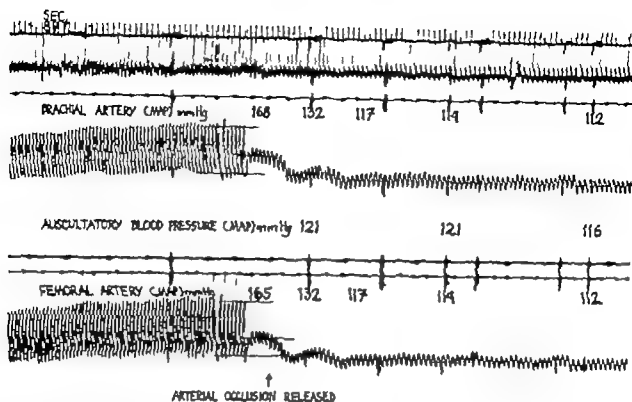


Fig 2 Model experiment showing simultaneously measured auscultatory blood pressure in the right arm and intra arterially recorded pressures in the left brachial (top) and femoral (bottom) artery during ischemia and after the arterial occlusion was released (arrow)

It might be questioned whether the blood pressure in the arm is representative for the blood pressure in the leg during maximal vasodilatation in the calf. In order to establish the relation of indirect blood pressure in the arm to intra arterial recordings of blood pressure in the brachial and femoral arteries a model experiment was performed. In four patients with resting blood pressure varying within a wide range catheters were inserted in the brachial and femoral arteries. Simultaneous auscultatory blood pressure in the arm was then compared to intra arterial pressures during the usual experimental conditions to produce maximal vasodilatation in the calf. A curve from this model experiment on one patient representative also for the other three patients is illustrated in Fig 2.

Intra arterially measured Blood pressure simultaneous in the brachial and femoral arteries showed a very close resemblance. The indirect blood pressure showed

Table 111 Model experiment in four patients showing results from simultaneously measured auscultatory blood pressure in the right arm and int a arterially recorded pressures in the brachial and femoral arteries. In the measurements with 10 seconds desufflation of the blood pressure cuff was started at the moment of arterial occlusion release. In the measurements 10-15 seconds after release of arterial occlusion the blood pressure cuff was insufflated immediately after arterial occlusion release.

Patient	Blood pressure (MAP) mm Hg with in 10 sec after release of ar- terial occlusion			Blood pressure (MAP) mm Hg 10-15 sec after release of arterial occlusion		
	Auscultatory	1 a brachial	1 a femoral	Auscultatory	1 a brachial	1 a femoral
1	121	132	132	124	118	119
2	129	121	114	123	117	125
3	135	151	147	130	133	134
4	116	108	117	114	114	114

less good agreement when directly recorded especially within 10 seconds after release of the arterial occlusion in the leg. It was also observed that immediately after the arterial occlusion was released a rapid blood pressure reduction took place in both arteries (Fig. 2). This blood pressure reduction occurred during about 5 seconds and a subsequently slower blood pressure reduction was observed during 30-60 seconds when the blood pressure in the femoral and brachial arteries was reduced towards resting values. When direct and indirect blood pressures were compared 10-15 seconds after release of the arterial occlusion better agreement was observed (Table 111).

It is obvious that the resistance calculated from blood flow and blood pressure might be biased if the indirect blood pressure in the artery is measured during the phase of rapid reduction. However, this could not be the case in the present study on patients refractory (Group A) and responding to the apy (Group B) since the blood pressure simultaneous with the maximal flow and termination was not measured earlier than 10 seconds after the release of the arterial occlusion when the blood pressure in the brachial and femoral artery was relatively stable. A systematic equality between the indirect and direct blood pressure measurements was observed in the present study.

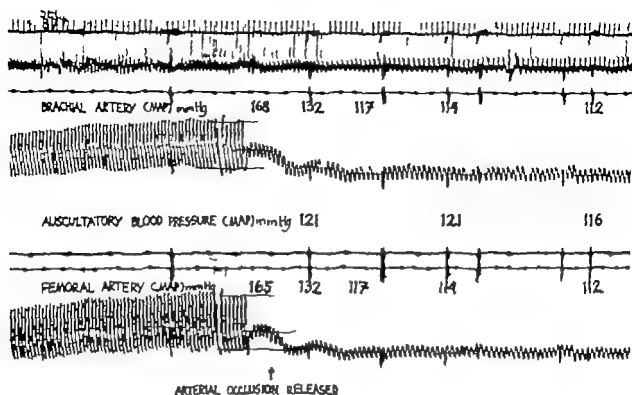


Fig 2 Model experiment showing simultaneously measured auscultatory blood pressure in the right arm and intra arterially recorded pressures in the left brachial (top) and femoral (bottom) artery during ischemia and after the arterial occlusion was released (arrow)

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Intra arterially measured blood pressure simultaneous in the brachial and femoral artery showed close resemblance. The indirect blood pressure showed

cardiac output determinations and during hand blood flow determinations at rest ($r = 0.75$ $p < 0.001$)

To test the variability of the blood flow resistance in the hand at maximal vasodilatation three consecutive determinations on twenty-four normotensive patients were used. The correlation coefficient was 0.83. No systematic change in resistance was observed in the consecutive recordings.

both patient groups in this study. The correlation was good ($r = 0.88$) (Fig 3). Consequently the error of the indirect blood pressure measurements should affect all patients equally and the resistance of vascular blood flow in the calf should be equally overestimated in both groups.

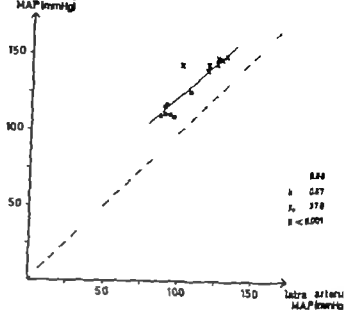


Fig 3 Correlation between simultaneously measured intra-arterial and auscultatory blood pressures. x = patients refractory to treatment, o = patients responding to treatment.

To test the variability of the blood flow resistance during maximal vasodilatation in the calf, three consecutive determinations in the twenty patients studied were used. The variation coefficient was 9.7%. No systematic change in resistance was observed in the consecutive recordings.

Hand

In the present series of twenty patients, calculations of vascular resistance in the hand were based on plethysmographic blood flow determinations and simultaneous intra-arterial blood pressure recordings in the contralateral brachial artery. The patients had both hands in water plethysmographs. During maximal flow experiments, the same temperature (43°C) and arterial occlusion time were applied bilaterally. The patients were instructed to exercise both hands simultaneously. Consequently, after these procedures, the blood pressure during hyperemic flow would be equally affected in both brachial arteries. The moments for blood flow determinations were marked on the blood pressure curves to ensure simultaneous recordings. The intra-arterial mean blood pressure during cardiac output determinations was 106.6 ± 16.0 mm Hg ($x \pm \text{SD}$) in the twenty patients studied and during hand blood flow determinations at rest 106.8 ± 14.3 mm Hg ($x \pm \text{SD}$). There was a significant correlation between mean arterial pressure during

another ten patients various renoparenchymal diseases were found. Renal vascular hypertension was found in four patients. Seven cases of unilateral hydronephrosis were detected. In addition there was one case of aortic coarctation, one of primary aldosteronism and two of primary hyperparathyroidism. In 29/40 (73 %) cases the diagnosis was made from the patient's history. In two cases did the investigations lead to a clinical interaction.

BLOOD PRESSURE REDUCTION (P per 11)

Fig. 4 presents the distribution of blood pressure in treated and untreated patients at the screening examination, at the third visit to the hypertension clinic and after one (n=646) and two years (n=624) treatment. The discrepancy in number was caused by patients who were lost to follow-up. The distribution of systolic blood pressures and diastolic blood pressures initially and after one and two years of treatment was gradually shifted to the left. The proportion of patients with systolic blood pressure or diastolic blood pressure above 160 or 95 mm Hg decreased significantly from 68 % (SBP) and 84 % (DBP) initially at the hypertension clinic to 33 % (SBP) and 5 % (DBP) after two years treatment. Correspondingly the proportion of subjects with systolic blood pressures above 170 or diastolic blood pressures above 105 mm Hg decreased from 47 % (SBP) and 53 % (DBP) to 17 % (SBP) and 17 % (DBP). The average blood pressure also decreased from 169/106 mm Hg initially to 153/96 mm Hg after two years treatment.

When only patients on drug treatment at the second annual examination were included (n=393) there was a significant reduction of systolic (15 mm Hg) and diastolic blood pressure (10 mm Hg) during two years follow-up (Table V). The major part of the reduction was achieved during the first year.

During the third year of follow-up the blood pressure control was studied in the 108 patients with diastolic blood pressure exceeding 105 mm Hg at the second annual examination. In 51/108 (47 %) patients a blood pressure below 95 mm Hg was achieved (Table VI). In 33/108 patients (30 %) the adequate control was achieved without changes (increasing doses or additional drugs) in therapy but in 18/108 (17 %) no change in therapy was needed. In 5/108 (5 %) patients the blood pressure control was inadequate.

RESULTS

PREVALENCE OF HYPERTENSION (Paper 1)

Of the 7455 men who were examined at the screening 1159 (16 %) had blood pressures above 175 mm Hg (SBP) or 115 mm Hg (DBP) or both or were receiving antihypertensive treatment. After exclusion of patients with blood pressure below the cut off points at the re examination 835 (11.2 %) were considered hypertensive and referred to follow-up (Table 1).

PREVALENCE OF SECONDARY HYPERTENSION (Paper 1)

Of the 686 patients investigated at the hypertension clinic 40 patients (5.8 %) were shown or suspected to have a specific cause of hypertension (Table IV). Fifteen patients had chronic glomerulonephritis and in

Table IV The frequency of diseases possibly causing secondary hypertension (n=686)

	n	%
Renoparenchymal disease	25	3.5
Chronic glomerulonephritis	15	
Renal tuberculosis	4	
Gouty nephropathy	3	
Renal dysplasia	2	
Analgesic nephropathy	1	
Renovascular disease	4	0.6
Aortic coarctation	1	
Primary aldosteronism	1	
Primary hyperparathyroidism	2	
Unilateral hydronephrosis	7	1.0
Total	40	5.8

ble V Means () and standard deviations (Sx) of systolic (SBP) and diastolic blood pressure (DBP) at screening initially at the hypertension clinic and after one and two years treatment. Only patients followed during the whole observation period and with antihypertensive therapy are included (n = 589)

	SBP		DBP	
	x	Sx	x	Sx
Screening	184	19.1	114	12.0
Initially at the clinic	168	20.1	106	12.4
After one year	158	18.2	97	9.8
After two years	153	17.7	96	9.8

Table VI Blood pressure control (diastolic blood pressure ≤ 95 mm Hg) during the third year of patients with diastolic blood pressure exceeding 105 mm Hg at the second annual examination (n = 108)

	No	%
Adequate blood pressure control after the therapy change	33	30
Adequate blood pressure control without the therapy change	18	17
Inadequate blood pressure control	25	23
Refused to participate	20	19
Lost to follow-up	8	7
Dead	4	4

In spite of dose increase efforts to find suitable drug combinations generally became the past it could not tolerate the medication or showed poor cooperability. 120/108 (19 %) patients considered adequately medicated with the drug as well as showing good cooperability the blood pressure control was still poor. Eight patients in this group were lost to follow-up and four patients died during the third year.

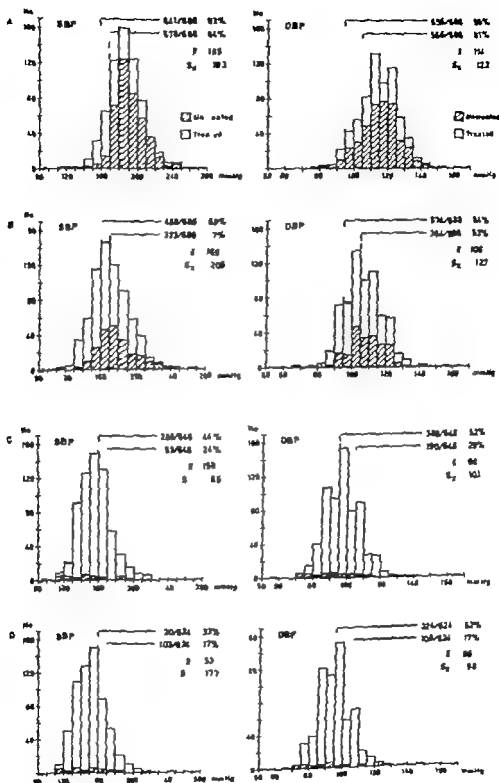


Fig 4 Systolic and diastolic blood pressure distribution for treated and untreated patients at screening (A) initially at the hypertension clinic (B) and two years treatment (D) and after one (C) and two years treatment (D) are also given as well as the frequency of patients exceeding 160 and 170 mm Hg (SBP) and 95 and 105 mm Hg (DBP) respectively

Seventeen patients (17/473 = 4 %) had to discontinue β adrenergic blocking treatment during the first year. In five patients this was due to vivid dreams or insomnia. Pulmonary obstructive symptoms were the reasons in four patients and gastrointestinal symptoms in another two. One patient developed bradycardia and another experienced worsened symptoms of intermittent claudication. Four patients had unspecific symptoms and wanted to change the β receptor blocking drug.

Two patients developed symptoms of arthritis while on hydroxyzine, but none had a true SLE like syndrome with positive titres for antinuclear factor.

In 34 patients during the first year, the side-effects causing withdrawal were caused by other drugs. The number of patients on each drug was, however, too low to make a rational analysis meaningful.

During the second year, nine patients (9/319 = 3 %) on diuretics and six patients (6/484 = 1 %) on β adrenergic blocking drugs had to discontinue treatment (Table VII).

PATIENT ADHERENCE

Twenty-nine patients (4.2 %) refused to participate or were lost to follow-up without known reason (Table VIII). Another 33 patients (4.8 %) were not followed during the entire observation period of two years. Eleven patients preferred treatment by other physicians, eleven left the city and eleven died.

Table VIII: Number of patients and reasons for drop-out during two years at the hypertension clinic (n = 686)

	First year		Second year	
	No.	%	No.	%
Refused/unknown reason	18	2.6	11	1.6
Preferred treatment by other physicians	9		2	
Moved	7		4	
Died	6		5	
Total	40	5.8	22	3.2

ADVERSE EFFECTS

In 90/610 (15 %) patients during the first year and in 15/589 (3 %) patients during the second year an antihypertensive agent had to be discontinued because of adverse effects considered caused by the drug. In another twelve cases (eight during the first and four during the second year) the relationship between the drugs and the adverse effects was considered improbable. These twelve cases were not included in the analysis below.

The reason for withdrawal concerning the three antihypertensive agents most commonly used during the first and second year are listed in Table VII. During the first year thirty seven patients (37/312 = 12 %) had to discontinue their diuretic treatment. Low levels of S-potassium in spite of potassium supplement was the most common reason. Seven patients developed S-urate levels exceeding 500 $\mu\text{mol/l}$ and one patient developed clinically overt gout considered secondary to the diuretic treatment. Four patients had to discontinue the treatment because of gastrointestinal symptoms and four developed clinically overt diabetes mellitus with glucosuria. Three patients complained of unspecific symptoms and wanted to change the diuretic drug.

Table VII Frequency of adverse effects causing withdrawal of the drug during the first and second year of treatment

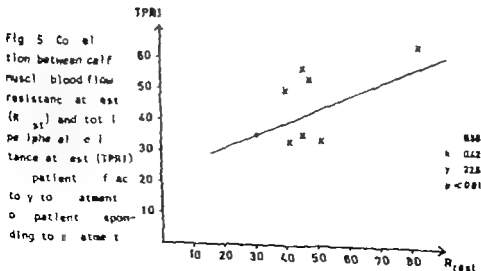
	First year		Second year	
	No	%	No	%
<u>Diuretics</u>	37/312	12	9/319	3
Diabetes mellitus	4		1	
Gastrointestinal symptoms	4	4	1	
Gouty arthritis	1		2	1
Others	3			
S-potassium < 3.4 mmol/l	18	8	4	
S-urate > 500 $\mu\text{mol/l}$	7		1	2
<u>β-adrenergic blocking drugs</u>	17/473	4	6/484	1
Sleep disturbances	5		3	
Pulmonary obstructive symptoms	4		1	
Gastrointestinal symptoms	2		1	
Bradycardia	1		1	
Intermittent claudication	1			
Others	4			
<u>Hydralazine</u>	2/207	1	0/225	0
Arthritis	2		0	

Central hemodynamic investigations

As illustrated in Table X the mean intra arterially recorded blood pressure differed significantly between refractory patients and the responders ($p = 0.01$). The cardiac index, heart rate and stroke volume index did not differ between the groups. Consequently total peripheral resistance index in the refractory hypertensive patients was significantly increased ($p = 0.05$).

Regional vascular resistance

The results from the blood flow determinations in the calf are shown in Table XI. The average blood flow at rest was similar in refractory and responding patients but the resistance at rest was significantly increased in the refractory group ($p = 0.01$). After a arterial occlusion and muscle work the flow increased 15 fold but there was no difference between the groups in this respect. The calf latence to maximal dilatation (R_{ml}) however was significantly increased in the refractory patients ($p = 0.05$). No difference between the groups concerning vascular tone at rest (R_{est}/R_{ml}) was found. There was a statistically significant correlation between total peripheral resistance index calculated from cardiac output and mean arterial blood pressure and blood flow resistance at rest ($r = 0.56$; $p = 0.01$) (Fig. 5).



HEMODYNAMIC CHARACTERISTICS IN PATIENTS REFRACTORY TO TRIPLE DRUG TREATMENT (Paper III)

Plasma volume

The hematocrits did not differ between refractory patients and responders and consequently differences in total blood volume were reflected by the plasma volume (Table IX). There was no significant difference between the groups either concerning total plasma volume or plasma volume per cm body height, but the refractory patients tended to have slightly higher values. Furthermore, no significant correlation was found between plasma volume per cm body height and indirect mean arterial blood pressure ($r = 0.36$ n.s.) or intra arterially recorded mean arterial blood pressure ($r = 0.42$ n.s.).

Table IX Total plasma volume and plasma volume per cm height in refractory patients (Group A) and patients responding to therapy (Group B)

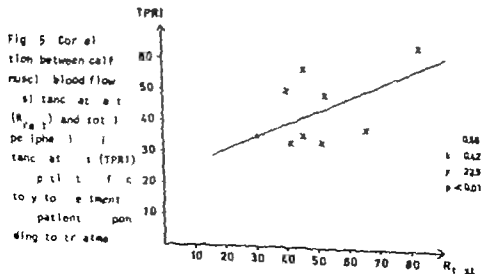
Patients	Plasma volume (l)		Plasma volume (ml/cm)	
	A	B	A	B
KR AM	3.7	2.8	21.4	15.6
KEW SG	3.1	2.7	17.8	15.4
KES SM	3.1	3.7	18.0	20.7
LW TB	4.8	2.4	26.8	14.0
EL HA	2.6	2	14.8	12.6
EE OR	3.6	3.1	20.7	17.7
BN BGH	3.6	4.0	20.2	21.7
OK DE	3.5	3.2	19.6	18.9
EA SW	3.4	2.9	19.2	16.4
RS EG	3.3	3.0	19.0	17.1
Mean	3.47	3.00	19.75	17.01
SD	0.57	0.55	3.09	2.85
Significance	ns		ns	

Central hemodynamic investigations

As illustrated in Table X the mean intr arterially recorded blood pressure differed significantly between refractory patients and the responders ($p < 0.001$). The cardiac index, heart rate and stroke volume index did not differ between the groups. Consequently total peripheral resistance index in the refractory hypertensive patients was significantly increased ($p < 0.05$).

Regional vascular resistance

The results from the blood flow determinations in the calf are shown in Table XI. The average blood flow at rest was similar in refractory and responding patient but the resistance at rest was significantly increased in the refractory group ($p < 0.01$). After a 100% occlusion and muscle work the flow increased 15 fold but there was no difference between the groups in this respect. The calculated resistance at maximal dilatation (R_{max}) however was significantly increased in the refractory patient ($p < 0.05$). No difference between the groups concerning muscular tone at rest (R_{rest}/P_{rest}) was found. There was a statistically significant correlation between total peripheral resistance index calculated from cardiac output and mean arterial blood pressure and blood flow resistance at rest ($r = 0.56$, $p < 0.01$) (Fig. 5).



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Table IX Total plasma volume and plasma volume per cm height in refractory patients (Group A) and patients responding to therapy (Group B)

Patients	Plasma volume (l)		Plasma volume (ml/cm)	
	A	B	A	B
KR AN	3.7	2.8	21.4	15.6
KEW SG	3.1	2.7	17.8	15.4
KES SM	3.1	3.7	18.0	20.7
LW TB	4.8	2.4	26.8	14.0
EL KA	2.6	2	14.8	12.6
EE OR	3.6	3.1	20.7	17.7
BN BGH	3.6	4.0	20.2	21.7
OK DE	3.5	3.2	19.6	18.9
EA SW	3.4	2.9	19.2	16.4
RS EG	3.3	3.0	19.0	17.1
Mean	3.47	3.00	19.75	17.01
SD	0.57	0.55	3.09	2.85
Significance	ns		ns	

T b t X l R e n g b l o o d f l o w n d i t c o a n d m e n a l b l o o d f l o w a n d l e t n c e m a l l
 i n g t o n t h e i f m u l s c i b e d i p a t i t f c t y (G o u p A) a n d
 p o n d m g (G o u p B) t t p l d r u g t z m e t

P client	R e i n g f l o w ml/100 ml		R e i n g s i s t a n (R _{sc}) PRU ₁₀₀		M a x i m a l f l o w ml/100 ml		R e i s t a n c e a t m a x i m a l f l o w (R _{min}) PRU ₁₀₀		R e s t i n g t o n (R _{rest} /R _{min})		
A	B	A	B	A	B	A	B	A	B	A	B
KA	AM	2 8	1 6	4 7 3	5 4 8	3 3 3	4 6 4	4 6	2 8	1 0 3	1 9 6
KEV	SG	3 1	2 9	4 4 9	3 4 1	4 1 6	4 8 2	3 3	2 3	1 3 6	1 4 8
RES	SH	2 7	3 3	4 5 0	2 9 5	3 9 0	4 4 2	4 1	2 4	1 1 0	1 2 3
LW	TB	3 0	4 0	3 9 4	2 3 8	3 7 1	3 1 6	3 7	3 0	1 0 6	7 9
EL	HA	1 6	3 1	8 1 3	3 1 3	2 7 7	3 0 5	5 0	3 3	1 6 3	9 5
EE	DM	2 4	2 4	5 2 1	4 1 0	5 2 2	3 8 3	2 4	2 7	2 1 7	1 5 2
BW	BGH	3 2	2 8	4 0 8	3 4 3	4 4 0	3 8 5	3 1	2 7	1 3 2	1 2 7
OK	DE	2 5	3 5	4 6 8	3 0 0	3 9 5	3 6 6	3 1	2 5	1 5 1	1 2 0
EA	SW	1 8	2 1	6 5 2	4 8 8	4 1 9	4 5 7	3 0	2 3	2 1 7	2 1 2
RS	EG	2 4	2 7	5 0 1	3 5 8	3 2 4	2 9 0	3 5	3 5	1 4 3	1 0 2
Means		2 6	2 8	5 1 3	3 6 3	3 9 0	3 8 9	3 6	2 8	1 4 8	1 3 5
SD		0 5	0 7	1 2 8	9 4	6 9	7 0	0 8	0 4	4 1	4 3
Significance		s		p 0 0 1		ns		p < 0 0 5		ns	

Table X Intra arterially recorded mean arterial pressure (MAP) cardiac Index (CI) heart rate (HR) stroke volume index (SVI) and calculated total peripheral resistance Index (TPRI) in refractory patients (Group A) and patients responding to triple drug therapy (Group B)

Patients		MAP mm Hg		CI (l/min m ²)		HR (beats/min)		SVI (ml/m ²)		TPRI (U/m ²)	
A	B	A	B	A	B	A	B	A	B	A	B
KR	AH	118	92	2.4	2.2	59	61	41	36	48.2	41.8
KEW	SG	130	100	2.3	2.3	58	59	40	39	56.5	43.5
KES	SH	124	106	3.5	3.7	55	58	64	64	35.4	28.6
LU	TB	124	86	2.5	2.8	67	67	37	42	49.6	30.7
EL	HA	127	86	2.0	2.1	54	58	37	36	63.5	41.0
EE	OR	111	106	2.3	2.4	74	48	31	50	48.3	44.2
BH	BCH	136	89	4.1	3.0	88	65	47	46	33.2	29.7
OK	DE	106	95	2.0	2.7	60	50	33	54	53.0	35.2
EA	SU	117	90	3.1	3.0	50	56	62	54	37.7	30.0
RS	EG	100	89	3.0	2.8	63	65	40	43	33.3	31.7
Mean		119	94	2.72	2.70	63	59	44	46	45.9	35.6
SD		11.2	7.6	0.7	0.5	11.2	6.3	11.4	9.1	10.5	6.3
Significance	p 0.001			ns	ns	ns	ns	ns	ns	p 0.05	

Table XII

Sympathoplegic blood flow and skin sympathetic tone of the hand vessels in patients refractory (Group A) and responding (Group B) to splenic artery atelectomy

Patient	Sympathoplegic flow ml/100 ml		Sympathoplegic tone (R _{symp}) PRU/100		Maximal flow ml/100 ml		No. distance at maximal flow (R _{min}) PRU/100		Sympathoplegic tone (R _{symp} /R _{min})	
	A	B	A	B	A	B	A	B	A	B
RA AM	9.4	13.4	13.1	6.7	35.2	45.6	4.2	2.5	3.1	2.7
KEV SG	9.8	13.7	14.3	8.3	30.0	39.3	5.4	2.8	2.6	3.0
RES SM	13.2	10.6	7.7	10.2	28.9	38.1	3.8	2.7	2.0	3.8
LW TB	10.0	9.1	12.7	9.9	34.5	30.4	3.7	2.7	3.4	3.7
EL HA	8.5	16.6	15.4	5.0	26.7	26.3	5.7	3.1	2.7	1.6
EE OR	7.5	13.2	14.2	7.8	29.4	28.4	4.6	3.8	3.1	2.1
BN BGH	14.6	18.0	7.4	5.0	48.4	47.5	2.7	2.0	2.7	2.5
OK DE	13.2	14.1	7.9	7.0	43.0	39.8	2.5	2.4	3.2	2.9
EA SV	13.9	15.9	6.6	6.1	35.1	27.9	3.9	3.2	1.9	1.9
RS EG	13.9	8.0	6.2	11.9	39.8	28.7	2.3	3.3	2.5	3.6
Mean	11.40	13.3	10.6	7.8	35.10	35.20	3.9	2.9	2.7	2.8
SD	2.6	3.2	3.7	2.3	6.9	7.8	1.2	0.5	0.5	0.8
Significance	ns		ns		ns		p < 0.05		ns	

The results from the determinations of hand blood flow are shown in Table XII. There was no difference at rest between the refractory patients and the responders as far as blood flow was concerned. Neither was there any significant difference between the groups concerning vascular resistance but it tended to be higher in the refractory patients. During maximal vasodilatation blood flow was almost identical in refractory and responding hypertensives. The resistance at maximal dilatation showed a significant increase in the refractory group ($p < 0.05$) compared to the responders. No difference was found concerning vascular tone. The correlation between total peripheral resistance and sympathoplegic resistance in the hand was statistically significant ($r = 0.63$, $p < 0.01$) (Fig. 6).

The ratio between treated mean arterial blood pressure for the two groups of patients ($145/115 = 1.3$) was almost the same as the ratio between resistance at maximal dilatation in the calf ($3.4/2.8 = 1.2$) and resistance at maximal dilatation in the hand vessels ($3.9/2.9 = 1.3$) as well as total peripheral resistance index ($46.0/35.6 = 1.3$).

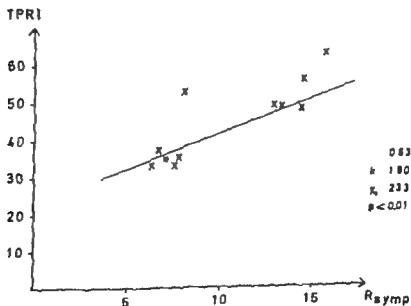


Fig. 6 Correlation between sympathoplegic hand blood flow resistance (R_{symp}) and total peripheral resistance at rest (TPRI).
x = patients refractory to treatment o = patients responding to treatment

The average blood flow in the calf at rest was significantly increased when the patients had minoxidil treatment ($p < 0.001$) and since the blood pressure decreased the calculated vascular resistance at rest was reduced ($p < 0.001$) (Table XIV). After arterial occlusion and muscle work the blood flow was increased to the same extent before and during minoxidil treatment. There was no change in resistance at maximal dilatation when the patients had minoxidil compared to when they were treated with hydralazine. There was a significant correlation between the blood pressure reduction and the change in resting blood flow ($r = 0.66$; $p < 0.05$) (Fig. 7). There was no correlation between the blood pressure reduction and change in resting resistance ($r = 0.47$ n.s.).

Adverse effects

One of the patients (BM) developed mild ankle oedema when receiving 20 mg daily of minoxidil. This sign disappeared when furosemide 40 mg daily was added to the total drug treatment. Eight patients had mild hypertrichosis of the face, trunk and legs. No patient had weight gain. No complaints of symptoms of any kind. No abnormal changes of coagulation. No changes in levels of serum aminase, serum electrolytes or serum lipids were observed. No tachycardia was noted. No patient wanted the drug to be discontinued when offered this option.

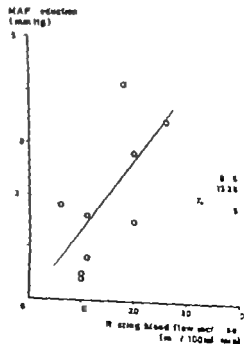


Fig. 7 Correlation between blood pressure reduction and resting blood flow increased during minoxidil treatment.

MINOXIDIL IN THE TRIPLE DRUG TREATMENT OF REFRACTORY HYPERTENSION

Blood pressure reduction

After 4-8 weeks on increasing doses of minoxidil all patients had a blood pressure reduction at the clinic considered adequate. All patients had blood pressures below 170/100 mm Hg and the mean arterial blood pressure reduction was 39 mm Hg ($p < 0.001$) when compared to pretreatment blood pressure. The reduction was 31 mm Hg ($p < 0.001$) when compared to blood pressure on triple drug treatment with hydralazine as the vasodilating agent. No significant change in heart rate was noted when the patients had minoxidil instead of hydralazine (Paper IV).

Plasma volume

The hematocrites did not differ between the examinations when the patients were on hydralazine or minoxidil. Consequently differences in blood volume were reflected by plasma volume. There was no change in total plasma volume or plasma volume per cm body height before and during minoxidil treatment (Table XIII). Furthermore, no correlation was found between the change in blood pressure and the change in total plasma volume per cm body height.

Table XIII Total plasma volume and plasma volume per cm body height before (B) and during (D) minoxidil treatment

Patients	Plasma volume (l)		Plasma volume (ml/cm)	
	B	D	B	D
KR	3.7	4.1	21.4	23.7
NEW	3.1	3.6	17.8	20.7
KES	3.1	3.6	18.0	20.9
LW	4.8	3.9	26.8	18.8
EL	2.6	3.4	14.8	19.4
EE	3.6	4.5	20.7	25.9
BN	3.6	4.0	20.2	22.5
OK	3.5	3.6	19.6	20.1
EA	3.4	3.5	19.2	19.8
RS	3.3	3.3	19.0	19.0
Mean	3.47	3.75	19.8	21.4
SD	0.57	0.36	3.09	2.15
p	ns		ns	

GENERAL DISCUSSION

THE SCREENING EXAMINATION

The patients in the present study were derived from the screening of a random third of the male population aged 47-54. The patient series was intended to be unselected and representative for the middle-aged male hypertensive population. Selection occurred however already in the screening phase as 25% of the invited subjects did not attend. This group has been shown to have a high mortality rate in diseases not associated with hypertension (88). Of the patients considered hypertensive at the screening examination 18% declined further follow-up (Table 1). Although it is conceivable that a large part of these patients were followed by other physicians, a considerable number of patients could not be examined in the present study thus limiting the possibility to generalize the results. However, the present patient series is considered to represent the total male middle-aged hypertensive population in a better manner than hospital series do. The participation rate in the present study is comparable to that in most other studies of this kind.

The cut-off points for hypertension at the screening examination may seem high. However, the arbitrary blood pressure levels were chosen to identify the patients in the upper decile of the blood pressure distribution. If therefore a high frequency of hypertension or cardiovascular involvement has been demonstrated in patients exceeding these levels (76) in a subsample of the present patient material the blood pressure level of 175/115 mm Hg in the afternoon corresponded to 162/101 mm Hg when measured in the morning (86) which is close to the WHO recommendations (83).

THE HYPERTENSION CLINIC

Organization

The new attitude concerning management of hypertension implies identification of all patients at risk standardized diagnostic work-up and the apy

Table XIV. Resting blood flow and resistance (R_{rest}) blood flow and resistance at maximal dilatation (R_{min}) and resting tone before (B) and during (D) minoxidil treatment

Patients	Resting flow ml/100 ml min		Resting resistance (R_{rest}) PRU ₁₀₀		Maximal flow ml/100 ml min		Resistance at maximal dilatation (R_{min}) PRU ₁₀₀		Resting tone (R_{rest})/(R_{min})	
	B	D	B	D	B	D	B	D	B	D
KR	2.6	4.1	47.3	28.0	33.3	31.0	4.6	4.2	10.3	6.7
KEV	3.1	4.9	44.9	24.3	41.6	34.6	3.3	3.7	13.6	6.6
KES	2.7	4.7	45.0	23.6	39.0	46.3	4.1	2.5	11.0	9.4
LW	3.0	5.6	39.4	17.9	37.1	46.3	3.7	2.6	10.6	6.9
EL	1.6	2.6	81.3	51.5	27.7	25.0	5.0	5.7	16.3	9.0
EE	2.4	3.4	52.1	32.9	52.2	40.0	2.4	2.8	19.3	11.8
BH	3.2	5.2	40.8	21.1	44.8	39.0	3.1	4.5	13.2	4.7
OK	2.5	3.6	65.3	34.7	39.5	33.0	3.1	3.7	15.1	9.4
EA	1.8	2.4	65.3	41.3	41.9	38.1	3.0	2.8	21.7	14.8
RS	2.4	3.5	50.1	34.9	32.4	30.7	3.5	3.0	14.3	11.6
mean	2.6	4.0	51.3	31.03	39.0	36.4	3.58	3.55	14.5	9.1
SD	0.52	1.08	12.75	10.19	6.90	6.87	0.79	1.02	3.7	3.0
Significance	p 0.001		p 0.001		ns		ns		p < 0.001	

blood pressure measurements have been made but still a biologic variation as well as inter-observer and intra-observer variations can be expected

In the patient series described (n=686) the blood pressure measurements have been done at different times of the day (8 a.m. - 4 p.m.) This would imply that a patient could be examined at 8 o'clock at one visit and at 3 o'clock at another. However, the annual blood pressure measurements used for comparisons were done in association with a visit to the physician and at fairly the same time of the day each year. Minor environmental factors affecting the results are difficult to avoid during clinical circumstances. There is no reason to believe, however, that such environmental fluctuations would result in better or lower blood pressure results.

Ideally, the same nurse should examine the same patient on every occasion but for practical reasons this has been impossible. Consequently, an inter-observer variation must be present. This variation, however, is considered minor as judged from inter-observer differences from simultaneous measurements performed. The same three nurses constituted the staff during the observation period. Repeated blood pressure measurements by the same observers also have good reproducibility.

Blood pressure control

The reduction of systolic and diastolic blood pressure between screening and the first measurement is the type of selection criterion (Table V) was anticipated for several reasons. Firstly, a considerable proportion of patients were already in the initial phase of antihypertensive treatment on their third visit to the clinic. Secondly, it is well known that the blood pressure gradually decreases without therapy during repeated measurements (17). Thirdly, methodological differences such as time of the day and position and a statistical phenomenon like regression towards the mean might explain some of the reduction. With the first fact in mind, the blood pressure reduction achieved during the first year might be considered undistorted but is nonetheless mainly a result of the antihypertensive drug treatment. After another year, the blood pressure level was maintained or even reduced somewhat. The results regarding blood pressure reduction during the first two years were not statistically significant. After the second year, 52% of the patients still had a diastolic blood

and a responsibility for a continuous follow-up. In that sense the out patient hypertension clinic in the present study could be regarded as a model for the management of large scale hypertensive populations. This is possible only if nurses replace the physicians in several functions and if a standardized form for diagnostic work-up is followed. Thus the physician has the results from three blood pressure measurements and the data from the diagnostic work up at hand at his first appointment with the patient - a rational and time saving approach. After the initial phase of treatment the nurses have the responsibility for blood pressure check ups and advice to the patients regarding the therapy and possible side effects. This function is supervised by the physician.

It might be anticipated that the patients could feel repelled in an organization characterized by effectiveness and swiftness as described here. However the results concerning patient adherence contradict this assumption.

The initial experiences from the hypertension clinic leads to the conclusion that a team of one full time nurse, one part time secretary and one full-time or a few part-time physicians can cope with the management of about 500 hypertensive patients of the kind studied here.

It must be remembered that the problem of treatment of hypertension can not be solved by out patient hypertension clinics of the kind described here. The prevalence of hypertension is so high that the majority of patients must be managed by general physicians. However the methods used are simple and could probably have wider applicability.

Blood pressure measurements

The blood pressure in all individuals is known to fluctuate within a wide range. A considerable difference of the blood pressure level during sleep and during different daily activities as well as a rapid rise in blood pressure from certain stimuli like pain, fear and excitement is described (60 a). Consequently the great variability of the blood pressure is often a problem in the diagnosis of hypertension.

In the present study serious attempts to standardize the procedure of

other studies (1, 2, 73) especially as only 44 during the two years follow-up were true failures to comply (Table VIII). Low cost for medical services and drugs, easy accessibility to nurses and physicians and the fact that the clinic actively located and arranged new appointments for patient who failed to attend at scheduled visits are considered the main reasons for this encouraging result.

PREVALENCE OF SECONDARY HYPERTENSION

The prevalence of secondary hypertension was low (5.8%) and that of surgically curable cases even lower. The prevalence of secondary hypertension in this study was lower than other estimates (18-22, 33). The analysis is however the first one to be done in subjects derived from screening a total population. Furthermore only men aged 47-54 years were studied. The prevalence of secondary hypertension might be higher in women or in younger men. The results suggest however that in middle aged men extensive investigations aimed at detecting secondary hypertension are not necessary in those found to have hypertension at screening. In patients with hypertension referred to hospitals secondary hypertension is probably overrepresented and more extensive routine investigations might be justified. Renography as screening instrument for renovascular hypertension cannot be recommended. The prevalence of renovascular hypertension was low and there were many false-positive renograms (3). The results support the findings of cost-benefit analyses of urography and renography as screening instruments for renovascular hypertension (54) and of comparisons of surgical and medical treatment of renovascular hypertension (55). The tests studied led to surgery for two patients, either of whom was cured. The present results and those quoted above thus suggest that in planning for community control of hypertension secondary hypertension should not be sought with advanced investigative methods. Instead only patients whose history, physical status or routine test results suggest secondary hypertension should be submitted to further investigations. The remainder, more than 95%, should be given diligent treatment.

HEMODYNAMIC CHARACTERISTICS IN PATIENTS REFRACTORY TO TREATMENT

It has been shown for several antihypertensive drugs that resistance to hypotensive treatment is associated with sodium retention and expanded

pressure exceeding 95 mm Hg which was the therapeutic goal (Fig 4). However, acceptable control, e.g. diastolic blood pressure below 105 mm Hg was achieved in 83 % of the patients at the second annual examination.

The results of blood pressure control during the third year in the group with diastolic blood pressure above 105 mm Hg indicate that in certain patients (Table VI) the blood pressure elevation at the second annual examination was only a temporary increase at this visit, as a substantial reduction was observed without increment of the treatment. Furthermore, improved blood pressure control was achieved after dose increments in a minor group of patients also during the third year of follow-up. Thus, even in an unselected hypertensive population treated by physicians with a special interest in hypertension, a long period of treatment was often needed to achieve adequate blood pressure control.

Adverse drug effects

The frequency of side effects causing withdrawal of a drug during two years' treatment must be considered acceptable. In spite of this, the frequency of adverse effects during the first year emphasizes the need for frequent appointments with a nurse or a physician and a possibility of telephone contact in the early phase of antihypertensive treatment. On the other hand, fewer side effects were registered during the second year of treatment, indicating that less close supervision is necessary after the initial treatment phase.

The β -adrenergic adrenoreceptor blocking drugs caused fewer adverse effects than the diuretics (Table VII). This might, however, be due to the fact that diuretics were more often prescribed to patients with advanced hypertensive disease in whom a higher frequency of side effects per se might be anticipated. Furthermore, the frequency of adverse effects with diuretics would also have been considerably reduced if a somewhat lower level of K^+ potassium had been accepted.

Patient adherence

The drop out rate at the hypertension clinic, 6 % during the first year and 3 % during the second, can be considered low in comparison with

asomotor blockade (sympathoplegic tone Table XII) The higher vascular resistance in the refractory group can therefore hardly be explained by an increased vascular smooth muscle tone

The other possible explanation for an increased vascular resistance in hypertension is that of structural changes in the resistance vessels. It has been shown that such structural changes are of great hemodynamic importance in established hypertension in man and animal (24 67 81). To study the possible role of this factor as an explanation for the higher resistance in the refractory group compared to the responders blood flow resistance in the hand and in the calf at maximal dilatation was determined. As the methods used for dilatation of the hand and calf blood vessels seem to induce an almost complete relaxation of the smooth muscle cells in the resistance vessels (see page 27) the higher resistance in the hand and in the calf at maximal dilatation in the refractory group strongly indicated the existence of more severe structural vascular changes in the refractory group than in the responding group. It is most likely that the structural changes in these patients may be due to a relative wall thickening in the resistance vessels i.e. increased wall/lumen ratio (24 31 67 72 81). Theoretically the higher resistance at maximal dilatation in the refractory group could be due to more severe arteriosclerotic changes of the big arteries of those patients. The findings of no radial oscillometries in the legs and no difference in blood pressure between the left and right arm in responders and non responders contradict however more extensive arteriosclerotic changes in the brachial or femoral artery of both groups.

The more pronounced structural changes in the refractory patients compared to the responders could be explained by more pronounced changes in the non-responders before treatment. Alternatively the explanation could be that the structural changes though initially of the same severity were more sensible on blood pressure lowering therapy (52 68) in the responding group than in the refractory group.

MINOXIDIL IN THE TRIPLE DRUG TREATMENT OF REFRACTORY HYPERTENSION

Minoxidil is still under clinical investigation and has been shown to be associated with a diastolic right atrium lesions in dogs (15). However

extracellular fluid volume (19-23). In the present patient series there was no significant difference between the refractory and the responding group concerning the total plasma volume nor was there any significant difference between the groups when plasma volume was expressed in ml/cm body height - a more reliable and reproducible indicator of intravascular volume (8-73). A trend towards higher values in the refractory group could however be seen and the lack of significance may be due to the small number of patients (Table IX). On the other hand since cardiac output was the same in the two groups and since the increased volume is thought to act on blood pressure via an elevated cardiac output - a volume factor explaining the higher pressure in the refractory group seems unlikely.

All patients in both groups had a high and equal degree of β adrenoreceptor blockade as shown by isoproterenol stimulation. Furthermore no difference between refractory patients and responders were shown concerning cardiac output, heart rate or stroke volume (Table X). Consequently with a substantially higher arterial blood pressure the refractory patients had a significantly increased total peripheral resistance in relation to the responders. The hemodynamic pattern well known to characterize patients with established hypertension.

An increased vascular resistance could either be due to increased smooth muscle tone in the resistance vessels and/or to structural changes in these vessels (24-67). Increased vascular smooth muscle tone can have several explanations, i.e. a higher vasomotor nerve tone, increased amounts of circulating pressor substances, increased sensitivity to vasoconstrictor substances, electrolyte differences and/or differences in myogenic tone. In the present series the possibility also exists that the antihypertensive drugs affected vascular smooth muscle tone differently in the two groups. Concerning the vasodilator used, the dose of hydralazine was the same in the matched pairs. As all patients refractory to triple drug treatment were shown to be slow acetylators, they should have equal or higher plasma concentrations of the drug than their matched controls since five of those patients were fast acetylators. The peripheral hemodynamic studies indicated that vascular tone in the resting calf muscle was the same in the two groups (vascular tone at rest, Table XI) as was the vascular tone in the hand (skin) at rest during functional

CONCLUSIONS

In the present study it has been shown that

acceptable blood pressure reduction and good patient adherence can be achieved in a majority of hypertensive patients treated at an out-patient hypertension clinic

The prevalence of secondary hypertension in an unselected hypertensive population of middle aged men is low and simple diagnostic tests to identify patients with secondary form of hypertension seem sufficient;

patients refractory to triple drug treatment with diuretics, propranolol and hydralazine have increased aortic flow resistance probably caused by structural changes of the resistance vessel walls;

when patients refractory to conventional drugs were given a potent vasodilating agent, mild to an acceptable blood pressure reduction was achieved. The blood pressure reduction was caused by a decreased peripheral resistance leading to increased muscle blood flow; thus

the main reason for the poor hypotensive response in the refractory patient was an increased aortic resistance.

It was considered justified to use the drug in the present series of patients refractory to treatment as these patients probably had an impaired prognosis (4-80). The patients were checked frequently but no adverse effects from the treatment were observed except for mild hypertrichosis.

Sodium and water retention during minoxidil therapy have previously been described (34-36). No weight gain or plasma volume increase was noted in the present series (Table XIII) implying that in these patients the diuretic treatment was sufficient to counteract the expected plasma volume increase. However, all patients had normal renal function (Table II) and more potent diuretic therapy might be needed in patients with impaired renal function (49-59).

The β blocking therapy shown to induce an effective blockade of the β receptors by isoproterenol infusions in the patients studied was sufficient to prevent tachycardia anticipated from the minoxidil therapy (Paper IV). That minoxidil caused an increase in cardiac output in spite of β adrenoreceptor blockade can not be ruled out since cardiac output was not determined during minoxidil treatment. Blood flow to the calf during rest was increased with about 50 % (Table XIV). This finding might be explained by an increased cardiac output, the alternative explanation being an altered distribution of blood flow.

All patients previously refractory to treatment had a considerable blood pressure reduction when given minoxidil (Paper IV). This blood pressure reduction was positively correlated to the increase in calf muscle blood flow (Fig. 7) and in these patients minoxidil obviously had a vasodilating capacity superior to hydralazine (Table XIV). As no change in blood flow resistance at maximal vasodilatation was demonstrated after the blood pressure reduction with minoxidil, the vascular abnormality in these patients did not show a tendency to be reversible as has previously been demonstrated in animal studies (52) as well as in man (68). It must be emphasized, however, that the observation time was probably too short for such changes to take place.

Patient acceptance of minoxidil in this study was excellent and the drug can be regarded as an alternative in the combination treatment of refractory hypertension.

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Supplementum 618

STUDIES ON FOUR HEREDITARY BLOOD DISORDERS IN ICELAND

By Ólafur Jensson

FROM THE BLOOD BANK, STATE HOSPITAL,
LANDSPÍTALINN UNIVERSITY HOSPITAL,
REYKJAVÍK, ICELAND

Studies on Four Hereditary Blood Disorders in Iceland

By
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PRINTED IN ICELAND
FELAGSPRENTSMÍÐJAN HF REYKJAVÍK 1978

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Leiknæðing Hákóla Íslands hefur á fundi sínum
9 nóvember 1977 samþykkt að taka ritgerð þessa gilda
til doktorsprófs

Ólafur Bjarnason
deildarforseti

INTRODUCTION

I had the good luck of being enrolled as a postgraduate student for one year in a course in Clinical Pathology at the Royal Postgraduate Medical School at the Hamner-Smith Hospital in London 1949-1950. I am always thankful to Dr med Ólafur Bjarnason, professor of Pathology at The University of Iceland for arranging this. My second stroke of luck occurred when my desire to continue my postgraduate studies in haematology was fulfilled and I joined Professor J. H. Dillie found a place for me as a voluntary research worker in the Department of Haematology at the R.P.G.M.S. 1950-1951 under Professor Sir John's leadership. These two years at the Hamner-Smith Hospital were decisive for my later work at home in Iceland. On several occasions during the years of study when I have made pilgrimages to the R.P.G.M.S. Professor Sir John has made arrangements for me to review my work at different stages at departmental meetings and his chief in two pathological techniques. Mr L. H. Wallott even visited Iceland in 1960 to assist me with the investigations on von Willebrand's disease supported by the NDOO fund in London.

Investigation of these conditions reviewed in this paper hereditary elliptocytosis hereditary spherocytosis and von Willebrand's disease began in 1958 when the author was a consultant in haematology to the City Hospital, Reykjavik for several months. Chief of the medical department Dr med

Ólafur Th. Thordarson offered me valuable opportunities in the beginning which were to guide me onto a path in human genetics. My work in haematology since 1959 for this Hospital in Reykjavik. Chief of the medical department Dr Ólafur Bjarnason, has been most valuable in addition to my participation in diagnostic haematology in hospitals in and outside Reykjavik from 1959-1973. I can only express my thank to the numerous colleagues medical technologists and hospital secretaries who have shown understanding and given me much assistance in my research.

My main laboratory facilities have been my private laboratory for haematology and clinical cytology located in Dómur Ándica Reykjavik since 1963, which has offered diagnostic service to general practitioners specialists and hospitals from 1963.

As a director of the Blood Bank from 1st of March 1972 my position to carry on with research in human genetics has improved and given me and my collaborators more opportunities and facilities to extend previous studies and add new items to our research in this field.

I acknowledge the understanding and support of the Ministry of Health and Governing Committee of the State Hospitals (University Clinic) to me and my coworkers investigations.

This review has been accepted by the Medical faculty of the University of Iceland for a M.D. degree.

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Nine publications on these disorders are listed below and designated by Roman numerals which will be used for reference in the text

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HEREDITARY ELLIPTOCYTOSIS IN ICELAND

In 1964 the first 35 cases of hereditary elliptocytosis in Iceland were reported in the Icelandic Medical Journal (I) In this paper the main features of this hereditary disorders were reviewed with reference to several of the classical papers on this condition Pedigree data presented in this paper was later developed in more detail (III) Some 170 kindred were scanned for the elliptocytic trait in the first phase of work on the kindred under study most of whom could be found in a book (Genealogical record) published in 1951 (Árnadóttir)

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The pedigree of the Icelandic elliptocytic family (III) is shown in Fig 1 and its geographic origin on The Map of Iceland page 10

Linkage studies on the elliptocytic family (III)

As stated in the paper (III) 1967 data on linkage was collected from over 100 individuals belonging to 38 sibships in the elliptocytotic family Professor J H Edwards suggested referring samples to Professor Harry Harris and Dr E B Robson at the Galton Laboratory and Dr T E Claghorn at the North London Blood Transfusion Centre Edware Middlesex who determined many marker systems on blood samples from the family members sent by air from Iceland to London The marker systems investigated are shown on 2 punchcards (Fig 2) which were used for the linkage data collected

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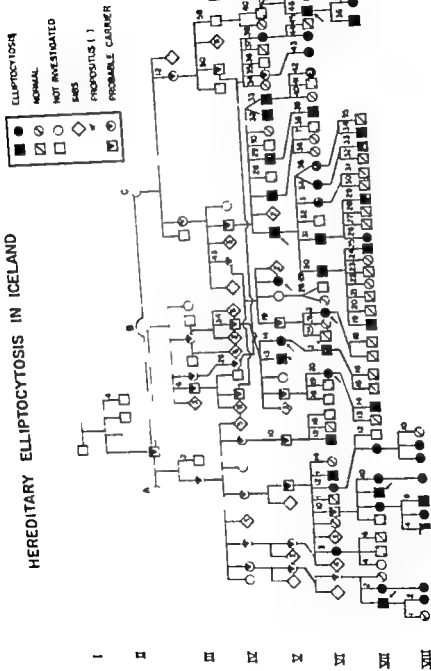


Fig 1 Pedigree of the family

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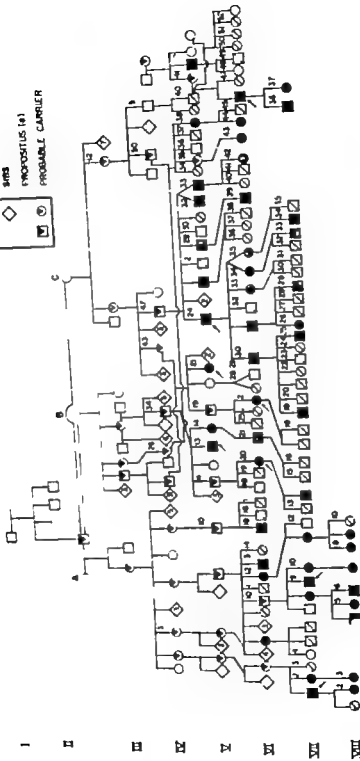
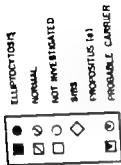


Fig 1 Pedigree of the family

The analysis of the linkage data was done on 23rd of December 1974 by Professor J H Edwards who used his linkage programme and the computer in Leiden by

courtesy of Professor Dr J J van Rood, Dept. of Immunohaematology University Hospital Leiden Holland

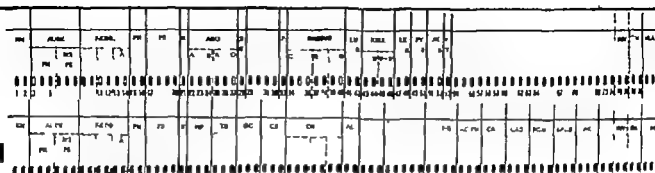


Fig 2 Card I 121 identification 2253 blood group data.

Card II 121 identification 2278 biochemical genetic markers

The 11 loci analysed in 29 suitable elliptocytic family units were ABO MNS P Rh Lewis bloodgroup systems acid phosphatase peptidases phosphoglucomutase 1 adenyate kinase and haptoglobins

The results (see Lod scores table 1) show no linkage between elliptocytosis locus in this family and other marker systems analysed

It has been known since 1953 (Goodall Hendry Lawler and Stephan 1953 Marshall Bird Bairley and Beckner 1954 and Morton 1956) that at least two varieties of hereditary elliptocytosis exist, one which is linked to the Rh blood group system locus on chromosome No 1 and another which is not linked to the Rh blood group locus The Icelandic elliptocytosis family is thus a good example of the latter non-linked variety

Recombination fraction	0.1	0.3	No. of scoring families
ABO	-0.868	-0.145	4
MNS	-0.413	-0.006	6
P	-0.462	-0.0	11
Rh	1.399	-0.189	5
Haptoglobin	0.068	0.032	2
Phospho-glucomutase 1	-0.041	-0.010	1
Acid phosphatase	1.714	-0.276	7
Adenyate kinase	0.013	0.093	2

Table 1

Results of linkage analysis of various loci and elliptocytosis

Results in Lods Close linkage with AcPh and Rhesus excluded

ALGERIAN ICELANDIC FAMILY WITH HEREDITARY ELLIPTOCYTOSIS

An Algerian-Icelandic family with hereditary elliptocytosis, reported in The Icelandic Medical Journal 1964 (11) is reviewed in some detail because it has not been published in English.

The proband, a boy was at the age of 3 months when diagnosis of elliptocytosis was made. His 2½ year old sister and his

mother had morphologically normal red corpuscle. The father an Algerian, had elliptocytosis, but no haematological changes indicating haemolysis. Test for sickling (wet wax sealed preparation) was negative in all four members of the family.

The results of haematological investigation made on this family are recorded in

Table 1

Haematological findings in the Algerian-Icelandic family. Three observations on the son in the 1st year

Family	Age	Condition	Hb g/100 ml	Haemato- crit %	RBC	MCV	MCHC	Reticulo- cytes %
	27	Elliptocytosis	14.9	45	5.40	83	33	0.8
	24	Normal	10.8	34	3.72	81	31	0.7
Daughter	2½	Normal	11.1	38	—	—	31	0.9
Son	3/12	Elliptocytosis	9.3	33	3.15	104	28	5.5
Son	4/12		9.0	31	3.78	80	29	5.0
Son	10/12		10.2	33	4.50	75	31	6.4

Table 2

Blood groups and biochemical genetic markers of the Algerian-Icelandic family

Blood groups	Father	Mother	Daughter	Son
ABO	O	O	O	O
Rh	cDe/cde	cde/cde	cde/cde	cde/cde
MN Ss	MNss	MNss	MNss	NNss
P	++	++	++	++
Lutheran	—e	—e	—e	—e
Kell	neg	neg	neg	neg.
Lewis	a—b+	a+b—	a—b+	a—b+
Duffy	—e	—e	—e	—e
Kidd	+a	+a	+a	+a
Biochemical Markers				
Haptoglobin	2-2	1-1	2-1	2-1
Transferrin	C	C	C	C
Serum cholinesterase (U Usual)	U	U	U	U
Acid phosphatase	BA	B	BA	BA
Phosphogluconate dehydrogenase	A	A+B	A+B	A+B
Phosphoglucomutase	1	1	1	1

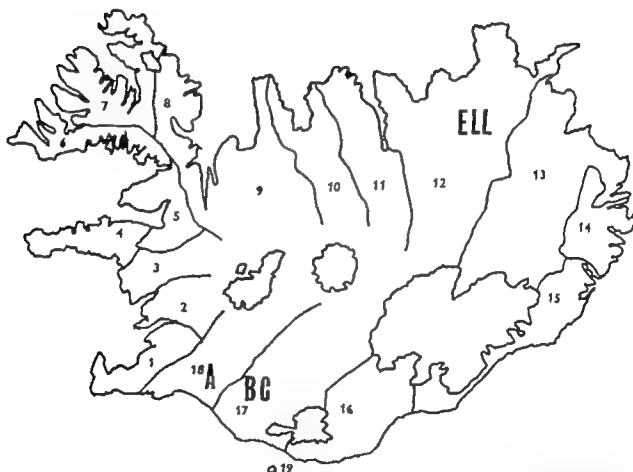
table 1 and blood groups and biochemical genetic marker systems done at MRC Human Biochemical Genetics Research Unit and Dept Biochemistry King's College London (Professor H Harris) and at North London Blood Transfusion Centre Edgware Middlesex (Dr T E Cleghorn) are shown in table 2

No linkage was suggested between the elliptocytosis main locus and the Rh blood

group system in this family and other markers tested for were uninformative

The affected boy had mild well compensated haemolytic anaemia during his first year. On several occasions from 1964-1974 his haematological values have been within normal range for his age

The author has no knowledge of the occurrence of hereditary elliptocytosis in Algeria



Map of Iceland

Geographic origin of the Icelandic elliptocytic family is found in county 12 ELL.

The homesteads of the von Willebrand's disease families A, B and C are found in counties 18 and 17

HEREDITARY SPHEROCYTOSIS

Studies on hereditary spherocytosis HS in Iceland (IV) contain clinical haematological and genetic data on 30 HS cases in 12 families. In addition 70 members of HS families have been surveyed haematologically. Symptoms and signs of HS have been searched for in all available family members. Genealogical information has been obtained from family members, genealogists and by study of many published Icelandic genealogical records.

The population of Iceland, which is Caucasian and mainly originated from Norway, Ireland and Scotland (Bjarnason et al. 1971) was just over 210,000 in December 1974.

HS is the most common hereditary haemolytic disorder in Caucasian peoples. Estimated prevalence of all humans is one in five thousand (1/5000) (Jacob 1972). Accordingly one would anticipate to find approximately 40 individuals with HS in the present population of Iceland. From 1945-1974 30 individuals with HS have been diagnosed, three of whom have died in 30 years covered by the present study.

As we have on record over 70 individuals with hereditary elliptocytosis which can with high probability be traced to single mutation in the past (see above) this haemolytic condition is the more common one of the two at this stage of knowledge.

Pedigree Studies

Seven HS families 1, 3, 11 and 9 show the relationship of 23 HS family members (Fig. 17). Families of seven HS cases have not been drawn up.

In families 2, 3 and 11 available genealogical information made it possible to draw up rather large pedigrees. These families form the basis for continued research on HS in Iceland along with the smaller HS families found.

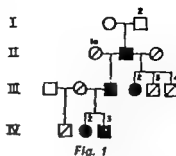


Fig. 1

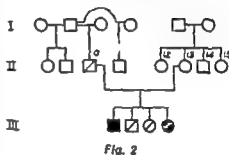


Fig. 2

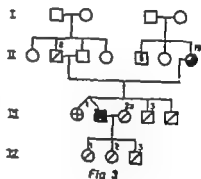


Fig. 3

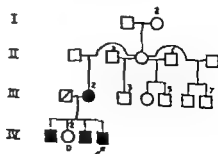


Fig. 4

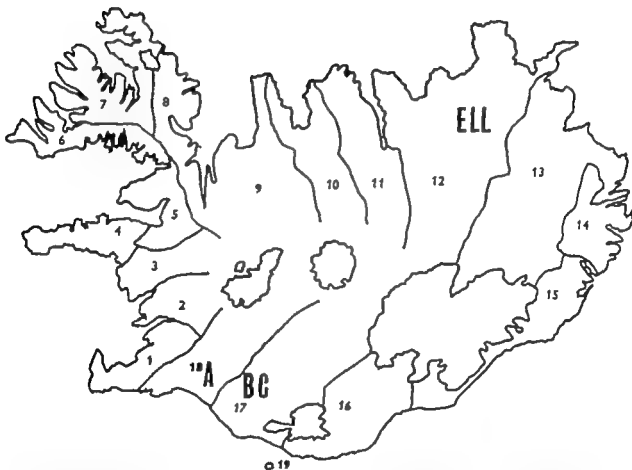
table 1 and blood groups and biochemical genetic marker systems done at M.R.C Human Biochemical Genetics Research Unit and Dept Biochemistry King's College London (Professor H Harris) and at North London Blood Transfusion Centre (Edgware Middlesex (Dr T E Cleghorn) are shown in table 2.

No linkage was suggested between the elliptocytosis main locus and the Rh blood

group system in this family and other markers tested for were uninformative.

The affected boy had mild, well compensated haemolytic anaemia during his first year. On several occasions from 1964-1974 his haematological values have been within normal range for his age.

The author has no knowledge of the occurrence of hereditary elliptocytosis in Algeria.



Map of Iceland

Geographic origin of the Icelandic elliptocytic family is found in county 12 ELL.

The homesteads of the von Willebrand's disease families A, B and C are found in counties 18 and 17.

HEREDITARY SPHEROCYTOSIS

Studies on hereditary spherocytosis HS in Iceland (IV) contain clinical haematological and genetic data on 30 HS cases in 12 families. In addition 70 members of HS families have been surveyed haematologically. Symptoms and signs of HS have been searched for in all available family members. Genealogical information has been obtained from family members, genealogists and by study of many published Icelandic genealogical records.

The population of Iceland which is Caucasian and mainly originated from Norway, Ireland and Scotland (Bjarnason et al 1971) as just over 216,000 in December 1974.

HS is the most common hereditary haemolytic disorder in Caucasian peoples. Estimated prevalence of all humans is one in five thousand (1/5000) (Jacob, 1972). Accordingly one would anticipate to find approximately 40 individuals with HS in the present population of Iceland. From 1945-1974 30 individuals with HS have been diagnosed three of whom have died in 30 years covered by the present study.

As we have on record over 70 individuals with hereditary elliptocytosis which can with high probability be traced to single mutation in the past (see above) this haemolytic condition is the more common one of the two at this stage of knowledge.

Pedigree Studies

Seven HS families 1, 5, 11 and 9 show the relationship of 23 HS family members (Fig. 1-7). Families of seven HS cases have not been drawn up.

In families 2, 3 and 11 available genealogical information made it possible to draw up rather large pedigrees. These families form the basis for continued research on HS in Iceland along with the smaller HS families found.

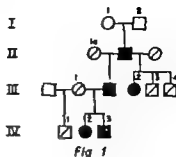


Fig. 1

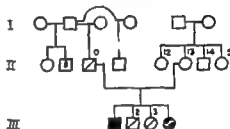


Fig. 2

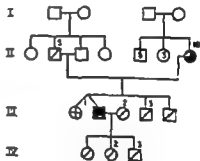


Fig. 3

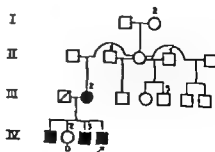
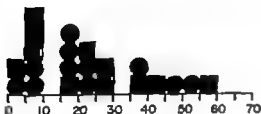


Fig. 4

**Table 1 Main Symptoms Signs and Laboratory Observations
In 22 Patients with HS before Splenectomy**

Case Nr./Family Nr	1/1	2/1	3/1	4/1	5/1	6/2	7/2	8/2	9/2	10/3	11/3	12/3
Sex	M	M	F	F	M	F	F	M	M	M	F	F
Age	41	18	7	2	4	46	22	5	4	58	10	19
Anaemia	+		+	+	+	+	+	+	+	+	+	
Jaundice	+		+	+	+	+	+	+	+	+	+	
Gallstones										+		
Splenomegaly	+		+			+			+	+	+	
Spherocytes	+	+	+	+	+	+	+	+	+	+	+	+
Reticulocytes %	10.7			17.0	13.0	14.2		10.0		40.0	1.0	3.5
Hemoglobin g/100 ml	12.4		7.5	4.3	6.3	8.0		9.3	11.0	9.6	3.0	11.5
Bilirubin mg/100 ml				2.5		1.5		3.65	1.5	2.25		1.2
Coombs test				—	—	—						
Osmotic fragility	+		+	+	+	+			+			+
Aplastic crisis						+					+	
Weight of Spleen	925	E.L.	255	80	170	580	300	300	160	775	1700	250
Accessory Spleen												

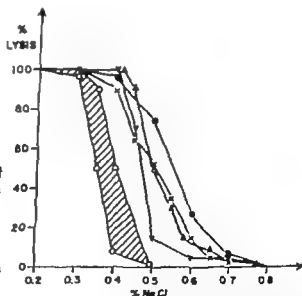
Enlarged



**Fig 8 Age (y) of HS patients at the time of
splenectomy Squares = males circles =
females**

	HS cases unoperated	Influenza Aplastic crisis
Females	5	1
Males	4	1 (died)
Total	9	4

Table 2 HS cases at risk during the Influenza 1957-1958



**Fig 9 Red cell fragility of 4 typical HS
patients in family 1 (Fig 1) and family 5
[Fig. 3] Striped area shows the normal
range**

14/4	16/5	17/6	18/7	19/8	25/10	26/11	27/11	28/11	29/12
E	M	F	M	F	M	F	M	M	F
22	35	7	6	35	27	51	27	21	15
+	+	+	+	+	+	+	+	+	+
+		+	+		+	+	+	+	
+					+	+		+	
	+				+	+	+	+	+
+	+	+	+	+		12.4	13.3	22.0	
77	55	10.3	11.0	7.0					
10.5	40	107	8.0	9.0	12.4	11.8	13.3	12.4	
2.3	17	4.8	2.1	1.2	3.4	1.9	3.8	6.4	
—	—	—	—	—	—	—	—	—	—
+	+		+	+					
+	+								
180	1150	340	200	260	1285	600	470	1400	375
		+	+				+	+	+



Table 1 Main Symptoms Signs and Laboratory Observations in 22 Patients with HS before Splenectomy

Case Nr./Family Nr	1/1	2/1	3/1	4/1	5/1	6/2	7/2	8/2	9/2	10/3	11/3	12/3
Sex	M	M	F	F	M	F	F	M	M	M	F	F
Age	41	18	7	2	4	46	22	5	4	58	18	19
Anæmia	+		+	+	+	+	+	+	+	+	+	
Jaundice	+		+	+	+	+	+	+	+	+	+	
Gallstones										+		
Splenomegaly	+		+			+			+	+	+	
Spherocytes	+	+	+	+	+	+	+	+	+	+	+	+
Reticulocytes %	10.7			17.0	13.0	14.2		10.0		40.0	1.0	3.5
Hæmoglobin g/100 ml	12.4		7.5	4.3	6.3	8.0		9.3	11.0	9.6	3.0	11.5
Bilirubin mg/100 ml				2.5		1.5		3.65	1.5	2.25		1.2
Coombs test				—	—	—						
Osmotic fragility	+		+	+	+	+			+			+
Aplastic crisis						+					+	
Weight of Spleen	925	E.L.	255	80	170	580	300	300	160	775	1 700	250
Accessory Spleen												
Enlarged												

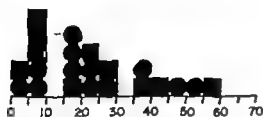


Fig 8 Age (y) of HS patients at the time of splenectomy Squares = males circles = females

	HS cases unoperated	Influenza Aplastic crisis
Females	5	3
Males	4	1 (died)
Total	9	4

Table 2 HS cases at risk during the Influenza 1957-1958.

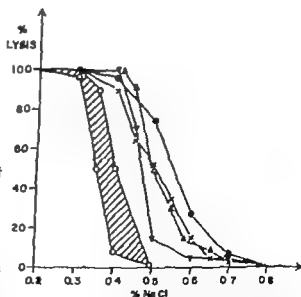


Fig 9 Red cell fragility of 4 typical HS patients in family 1 (Fig 1) and family 5 (Fig 3) Striped area shows the normal range

14/4	16/5	17/6	18/7	19/8	25/10	26/11	27/11	28/11	29/12
F	M	F	M	F	M	F	M	M	F
22	35	7	8	35	27	51	27	21	15
+	+	+	+	+	+	+	+	+	+
+		+	+		+	+	+	+	+
+						+		+	
	+				+	+		+	
+	+	+	+	+	+	+	+	+	+
7.7	5.5	10.3	11.0	7.0		12.4	13.3	22.0	
10.5	4.0	10.7	6.0	9.0	12.4	11.8	13.3	12.4	
2.3	1.7	4.8	2.1	1.2	3.4	1.9	3.8	6.4	
—	—	—	—	—	—	—	—	—	—
+	+		+	+					
+	+								
180	1 150	340	200	290	1 285	600	470	1 400	375
		+	+				+	+	+



Fig. 10 Map of Iceland. Geographic origin of 10 H8 families indicated by asterisks and Arabic numerals.

Clinical and Laboratory Data on HS

Of 30 HS cases diagnosed 22 have undergone splenectomy Fig 11. Serious consequences of delayed splenectomy associated with influenza epidemic 1957-1958 is shown in Table 2.

The main clinical symptoms signs and laboratory findings of the 30 HS cases are

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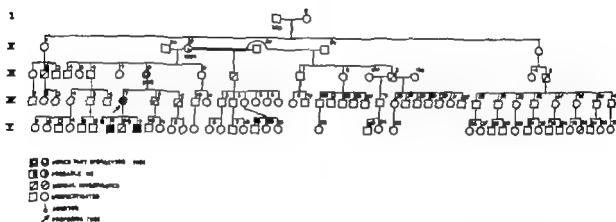


Fig 5 Family 2

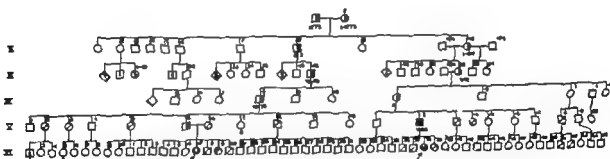


Fig 6 Family 3 Symbols as in Fig 5

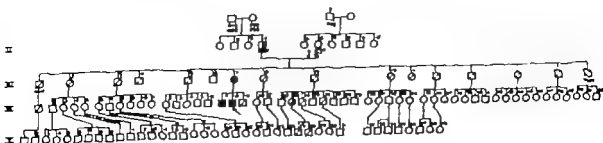


Fig 7 Family 11 Symbols as in Fig 5

STUDIES ON THE PELGER ANOMALY IN ICELAND

The first Icelandic family containing twelve members with Pelger anomaly were reported in 1963 (V).

As many members of the Pelger family had been living in a comparatively isolated district for several generations high frequency of the Pelger gene in the district was suspected. This led to a survey of 1197 of total of 1441 individuals (83 per cent) living there in 1964. No additional Pelger anomaly individuals were detected in the medical district.

In April 1975 a female born 1944 was diagnosed in a mental hospital in Reykjavík with Pelger anomaly. She and her father who had the anomaly had ancestors in common with the members of the Pelger family previously reported (VI).

Pedigree Studies

With the aid of two large Icelandic family records published (Jónsson 1953, Magnússon 1960) and a record of lawyers in Iceland (Jónsson 1963) it has been possible to trace the two branches of this family to common ancestors born 200 years ago (Fig. 1 pedigree drawing).

The homestead of the two family branches is shown in Fig. 2, the one detected in 1975 lives in the Eastfords.

American-Icelandic family with Pelger anomaly

The Pelger anomaly was found in another family in 1974 (VI). The mother an American married to an Icelandic and her youngest child of four a boy had the anomaly. The findings are shown in table 1 and 2.

Table 1 Results on the American-Icelandic Pelger family members.

Family member Year of birth	Father 1939	Mother 1944	Daughter 1962	Daughter 1964	Son 1965	Son 1970
<i>Hematologic Observations</i>						
Condition	Normal	Pelger	Normal	Normal	Normal	Pelger
ESR	3	2	6	5	7	3
Hemoglobin g/100 ml	15.0	12.0	13.0	13.4	13.0	9.8
Hematocrit %	45	40	37	40	39	31
MCHC	33	32	35	34	33	31
WBC	11800	7200	8500	6600	9400	5200
<i>Differential Count</i>						
Stab form %	3	37	2	4		9
Bilobar	23	32	28	28	22	27
Trilobar %	38	1	35	26	24	6
Quadrilobar %	4		8	2		
Lymphocytes %	28	28	27	34	41	54
Monocytes %	8	4	2	2	6	6
Eosinophils %	0	0	0	6	7	4
Basophils	0	0	0	0	0	0

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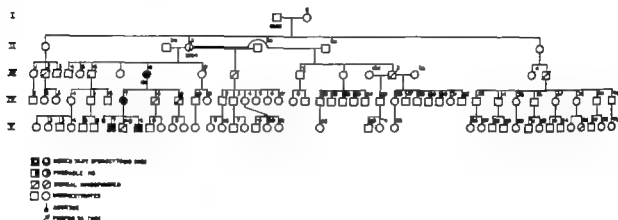


Fig 5 Family 2

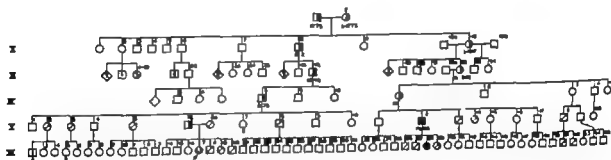


Fig 6 Family 3 Symbols as in Fig 5

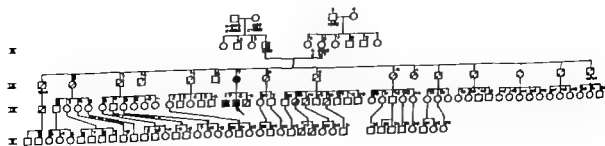


Fig 7 Family 11 Symbols as in Fig 5

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Table 1 Results on the American-Icelandic Pelger family members.

Family member Year of birth	Father 1939	Mother 1944	Daughter 1962	Daughter 1964	Son 1965	Son 1970
<i>Hematologic Observations</i>						
Condition	Normal	Pelger	Normal	Normal	Normal	Pelger
E.S.R.	3	2	6	8	7	3
Hemoglobin g/100 ml	15.0	12.0	13.0	13.4	13.0	9.8
Hematocrit %	45	40	37	40	39	31
MCHC	33	32	33	34	33	31
WBC	11600	7200	8500	6600	9400	5200
<i>Differential Count</i>						
Stab form %	3	37	2	4		9
Bilobar %	23	32	28	26	22	27
Trilobar %	38	1	33	26	24	11
Quadrilobar %	4		6	2		
Lymphocytes %	28	28	27	34	41	54
Monocytes %	6	4	2	2	6	6
Eosinophils %	0	0	0	8	7	4
Basophils	0	0	0	0	0	0

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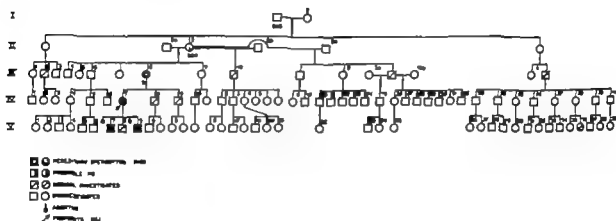


Fig 5 Family 2

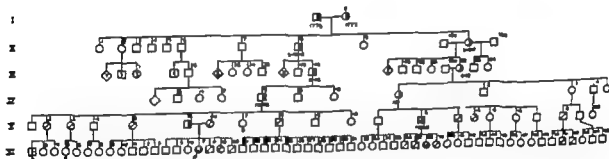


Fig 6 Family 3 Symbols as in Fig 5

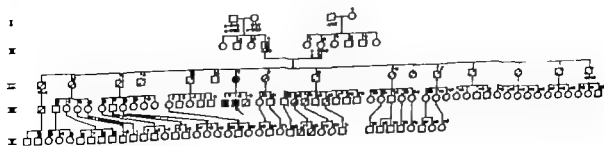


Fig 7 Family 11 Symbols as in Fig 5



Fig 2. — Map of Iceland. Homesteads of the two branches of the Petger family: P 1 detected in 1962 and P 2 in 1975.

Table 2 Results on the American-Icelandic Pelger family members.

Family member	Father	Mother	Daughter	Daughter	Son	Son
Year of birth	1939	1944	1962	1964	1965	1970
Condition	Normal	Pelger	Normal	Normal	Normal	Pelger
<i>Biochemical genetic markers</i>						
Serum esterases	usual	usual	usual	usual	usual	usual
Hæmoglobin	A	A	A	A	A	A
Haptoglobin	1-1	2-1	2-1	1-1	1-1	2-1
Transferrin	C	C	C	C	C	C
<i>Blood groups</i>						
ABO	O	A ₁ B	A ₁	B	A ₁	B
Rh	R ₂ r	R ₁ r	R ₁ r	rr	R ₂ r	rr
MNSs	MaNs	MaMs	MaMs	MaNs	MaMs	MaNs
Kell	neg	neg	neg	neg	neg	neg
Fy ^a	+	+	+	neg	+	+

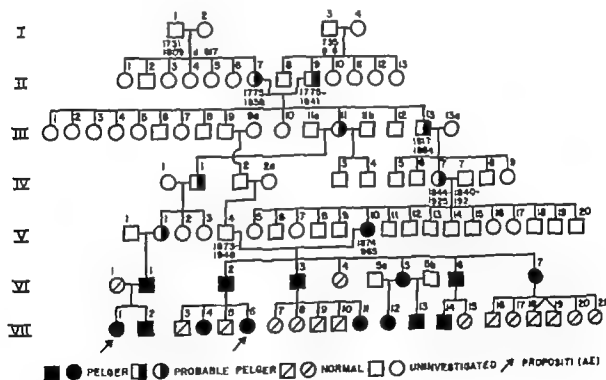


Fig 1 Pedigree of the Icelandic Pelger family



Fig 2. — Map of Iceland. Homesteads of the two branches of the Palger family: P 1 detected in 1982 and P 2 in 1975.

VON WILLEBRAND'S DISEASE IN ICELAND

In 1959 a boy was admitted to the Municipal Hospital in Reykjavik with bleeding tendency. His clinical history, his family history and a few laboratory tests done at that time pointed to von Willebrand's disease. Coagulation studies on this family had to be put off until 1969. In that year a second family originated from the same district as the first, was investigated due to similar symptoms and signs (VIII). The third family from the same district was brought to attention when an eleven year old girl was admitted to the University Clinic (Landspítaliinn) in July 1969 due to persistent vaginal bleeding for three weeks following her first menstruation (IX).

These families are illustrated in the pedigree drawings: family A, Fig 1 (VIII), family B, Fig 2 and family C, Fig 3 (IX).

The survey of relatives for clinical symptoms of bleeding tendency covered 120 individuals in Family A. Of these a total of 41 family members belonged to nine sibships which contained one parent affected. Results of coagulation tests on 15 members of Family A is recorded in table 1. Coagulation studies on two members in a branch of this family living in America performed by the late Judith G. Pool at the Stanford Medical Center in California and on two others done in Iceland showed disproportionate rise of factor VIII following infusion of factor VIII concentrate (VIII).

One of the members in Family A, III 12, who transmitted the von Willebrand's disease

gene to her children also had typical hereditary haemorrhagic telangiectasia lesions in the oral cavity and gastric mucosa (Quick 1974).

Of 80 family members of Family B and C surveyed for clinical symptoms of bleeding tendency 45 belonged to family units in which one of the parents and/or one or more sibling were affected. Results of coagulation tests done on 4 members in Family B and 10 members in Family C are shown in table 2. In Fig 4 factor VIII values found in 28 members of the families A, B and C are compared. The male members of three families manifest more severe clinical symptoms of bleeding than the females and they also tend to have lower levels of factor VIII. A total of 27 individuals have been ascertained as affected: 17 males and 10 females.

The variability in clinical symptoms and in the levels of factor VIII and in addition the uneven genetic ratio apparent, suggests that the mutant gene is present in latent form in some of the members of the three families studied.

It is thought probable that the mutant gene present in the three von Willebrand's disease families has originated from a common progenitor in the area. Efforts to find such a relationship by genealogical approach have not been successful yet. Geographic origin of the three families is shown on the map of Iceland, page 10.

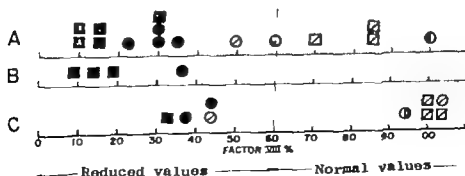


Fig 4 — Factor VIII values estimated in 28 individuals in families A, B and C

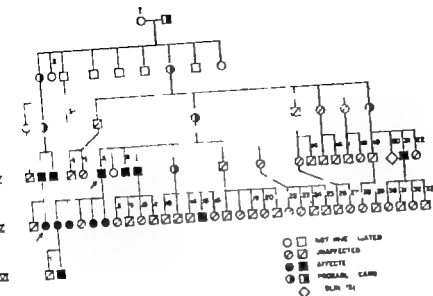


Fig 1 Family A.

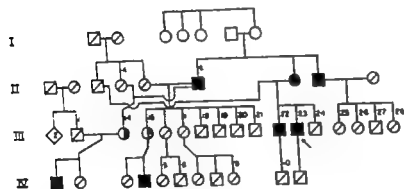


Fig 2 Family B.



Fig 3 Family C.

DISCUSSION

It is generally agreed that the four hereditary conditions studied hereditary elliptocytosis von Willebrand's disease the Pelger anomaly and hereditary spherocytosis (HS) show autosomal mode of inheritance (Wintrobe 1975 Britton 1974 Dacie 1960 Hardisty and Ingram 1965)

In each of these conditions Icelandic families have been studied who by genealogical information obtained indicate that the mutant genes causing these disorders and present in the investigated living family members has been transmitted through at least six generations or 150-200 years. The origin of these families has been traced to particular areas in Iceland. The relationship of affected family members and their common geographical origin is considered to suggest, that the same type of mutant gene is transmitted in these families. Direct biochemical methods explaining the precise nature of the abnormality caused by these mutant genes are presently not available for any of the four hereditary conditions studied. Therefore direct proof or disproof of the genetic homogeneity of the conditions under study is not possible.

Ascertainment of the affected family members is based on morphological features of the blood cell mainly (in hereditary elliptocytosis Pelger anomaly and HS) and abnormal plasma coagulation factors in the case of von Willebrand's disease.

Variants of each of these hereditary condition have been described by many workers indicating possible genetic heterogeneity. The demonstration of genetic heterogeneity of hereditary elliptocytosis by linkage studies referred to previously is the only proof of genetic heterogeneity in the four hereditary conditions studied.

Hereditary elliptocytosis

In the Icelandic family with elliptocytosis (III) not closely linked to the Rh blood group loci (Cf linkage studies) the morphological features of the red cell have made ascertainment easy. The expected one to one genetic ratio is fulfilled and the clinical findings in affected family members at various ages

indicate no apparent selection against the elliptocytic members or shortening of their life span.

The considerable variation in symptoms and signs of the affected family members is found both within the same sibship or between sibships in different branches of the elliptocytic family (III). The cause of this variation can be explained by non-genetical factors such as infection in some cases while in other cases genetic make-up is probably the main cause for this variation.

It has been suggested that hereditary elliptocytosis not linked to the Rhesus blood group system gave rise to more symptoms and signs of haemolysis than the Rh linked variety (Bannerman and Renwick, 1962).

Transient red cell morphological changes were studied in newborn male twins who both developed hyperbilirubinaemia on the first and second day after birth. One of the twins underwent exchange transfusion (Jensen et al 1978 unpublished observations). Their father has elliptocytosis (VI 48 see Icelandic family with elliptocytosis). Their red cell blood picture was very pleomorphic for the first 4-6 weeks but by the age of 3-4 months both twins had developed typical elliptocytic blood picture (Austin and DeForge 1969). Hereditary elliptocytosis as well as HS (Burman 1958) are among many hereditary red cell defects which can give rise to excessive neonatal jaundice in addition to haemolytic disease of the newborn due to immunological causes (Stamley Diamond 1957 Kostinas et al 1967 Oski 1974).

Hereditary spherocytosis (HS)

Apart from typical HS cases in relatively small families showing a dominant mode of inheritance (Meulengracht 1922 Race 1942 McKusick, 1968) three large HS families have been studied in whom typical HS cases have been diagnosed in different branches and thought to contain members with mild HS or HS in an undiagnosable form (Buttersworth et al 1950 Carruthers 1960 Dacie 1960). The mild or equivocal forms of HS are

commented on by Decle, 1960 p. 107 as follows. "Subsequent writers have attempted to overlook the possibility of the extremely mild forms described by Gärdsalen" (Gärdsalen 1922, 1925 1928 cf Decle's references 1960)

From the present study of families 2, and in particular 3 and 11 the presence of mild HS cases is highly suggestive

When family members in four generations belonging to HS families 2 and 11 (Table VI) are added up, half of the 168 individuals entered as "normal" or approximately 85, could theoretically be affected. Only 7 of the 9 members listed as affected have been diagnosed suffering from HS and two are entered (parents) as probably affected (IV)

The present data are suggestive of a much higher frequency of the HS gene or genes than is indicated by overtly affected HS cases admitted to hospitals. The hospital frequency shows probably only the tip of the iceberg and not the true HS gene frequency in the population. From the present study of families 2 and in particular 3 and 11 one is inclined to consider the prevalence of HS gene or genes mainly due to the presence of mild forms of HS which, by a presently unexplained mechanism, can occasionally cause severe typical forms of HS in one or more family units within the "mild" gene family

Admittedly only 36 of the 168 family members recorded in Table VI have been studied haematologically at this stage. But the results so far obtained (Table IV) and discussed above illustrate the difficulties in making ascertainment in extremely mild cases of HS by laboratory methods presently available

Peiger anomaly

Of the ambulatory patients scanned by one of us (O J) referred by practising family physicians and specialists approximately 90 are from Reykjavik and neighbouring municipalities and counties. Individuals from more distant places, over 50 km from Reykjavik form insignificant part of the number surveyed. A few inhabitants from the most remote counties in the north, east and south-east of Iceland are amongst the

40,000 scanned for the Peiger anomaly and recorded in Table 1 in VII.

In this century there has been a great movement of Icelanders from various rural parts of Iceland to the coastal villages and municipalities and in particular to the capital and surrounding areas in the south-west of Iceland

The representation of people from the north-eastern and eastern parts is probably less in the present population living in Reykjavik and the surrounding area, than from most other parts of Iceland. It is therefore probable that further scanning of individuals from the east and north-east part of the country will add new information as to the Peiger gene distribution and frequency in Iceland

On the basis of present results it is considered most probable that the Peiger anomaly gene has been present in Icelanders over 200 years at least. The scanning of relatively large sample of the population of Iceland makes it also possible, that the large Peiger family described contains the only mutation of this kind in Icelanders.

Distribution of the Peiger anomaly is worldwide and its frequency ranges from as high as 1 in 1000 persons to one in 10 000 (Johannsson, 1963; Witrobs, 1974).

Acquired Peiger anomaly has been described by many investigators (Johannsson, 1963) mainly associated with development of leukaemia. Such changes in the neutrophil leucocytes have been found in one Icelandic family associated with abnormal blood marrow clone (Kaur et al 1972).

Von Willebrand's disease (v W)

The variation in symptoms and signs and in the results of coagulation and bleeding tests in the v W Icelandic families studied is in accord with most other workers who have studied this condition in the last 20 years (Nilsson et al 1959 Rizza 1975 Nilsson and Holmberg 1973)

The application of the more recent laboratory methods in particular immunoelectrophoresis (Sides et al 1971 Zimmermann et al 1971 Rizza, 1975) make it possible to distinguish mild haemophilic

DISCUSSION

It is generally agreed that the four hereditary conditions studied hereditary elliptocytosis von Willebrand's disease the Pelger anomaly and hereditary spherocytosis (HS) show autosomal mode of inheritance (Wintrobe 1975 Britton 1974 Dacie 1960 Hardisty and Ingram 1965)

In each of these conditions Icelandic families have been studied who by genealogical information obtained indicate that the mutant genes causing these disorders and present in the investigated living family members has been transmitted through at least six generations or 150-200 years. The origin of these families has been traced to particular areas in Iceland. The relationship of affected family members and their common geographical origin is considered to suggest that the same type of mutant gene is transmitted in these families. Direct biochemical methods explaining the precise nature of the abnormality caused by these mutant genes are presently not available for any of the four hereditary conditions studied. Therefore direct proof or disproof of the genetic homogeneity of the conditions under study is not possible.

Ascertainment of the affected family members is based on morphological features of the blood cell mainly (in hereditary elliptocytosis Pelger anomaly and HS) and abnormal plasma coagulation factors in the case of von Willebrand's disease.

Variants of each of these hereditary condition have been described by many workers indicating possible genetic heterogeneity. The demonstration of genetic heterogeneity of hereditary elliptocytosis by linkage studies referred to previously is the only proof of genetic heterogeneity in the four hereditary conditions studied.

Hereditary elliptocytosis

In the Icelandic family with elliptocytosis (III) not closely linked to the Rh blood group loci (Cf linkage studies) the morphological features of the red cell have made ascertainment easy. The expected one to one genetic ratio is fulfilled and the clinical findings in affected family members at various ages

indicate no apparent selection against the elliptocytic members or shortening of their life span.

The considerable variation in symptoms and signs of the affected family members is found both within the same sibship or between sibships in different branches of the elliptocytic family (III). The cause of this variation can be explained by non-genetical factors such as infection in some cases while in other cases genetic make-up is probably the main cause for this variation.

It has been suggested that hereditary elliptocytosis not linked to the Rhesus blood group system gave rise to more symptoms and signs of haemolysis than the Rh linked variety (Bannermann and Ranwick 1962).

Transient red cell morphological changes were studied in newborn male twins who both developed hyperbilirubinaemia on the first and second day after birth. One of the twins underwent exchange transfusion (Jensen et al 1976 unpublished observations). Their father has elliptocytosis (VI 46 see Icelandic family with elliptocytosis). Their red cell blood picture was very pleomorphic for the first 4-6 weeks but by the age of 3-4 months both twins had developed typical elliptocytic blood picture (Austin and De-forges 1969). Hereditary elliptocytosis as well as HS (Burman 1958) are among many hereditary red cell defects which can give rise to excessive neonatal jaundice in addition to haemolytic disease of the newborn due to immunological causes (Stamey Diamond 1957 Kostina et al 1967 Oski 1974).

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vík area. Genealogical information indicates that the Pelger anomaly gene has been present in this family over 200 years.

The Pelger anomaly was diagnosed in 1974 in an American woman married to an Icelandic. One of their four children, a boy also has the anomaly.

Von Willebrand's disease

Three families with vW are reviewed. They include 27 affected individuals, 17 males and 10 females. Severe symptoms of bleeding predominate in the males, two of whom have died from haemorrhage. Typical lesions of hereditary haemorrhagic telangiectasia

were found in one female who was considered to be a carrier of the vW gene.

There is a reduced expressivity of the mutant gene, amounting to nonpenetrance mainly in the female members of the families. The clinical laboratory and hereditary findings in the vW families studied suggest much higher prevalence of the mutant vW gene than is indicated by clinically affected cases and cases ascertained by the laboratory tests used.

It is thought probable that the mutant gene present in the three families has originated from a common ancestor in a district which is common to the three families. Efforts to find relationship between them have been unsuccessful up to now.

ACKNOWLEDGEMENTS

I am much indebted to Professor John H Edwards, Department of Human Genetics, University of Birmingham, consultant to the Genetical Committee of the University of Iceland. Over the last 10 years his help has been invaluable to me and my co-workers studies on human genetics in Iceland. To my former teacher Sir John Dacie, Professor of Haematology at the Royal Postgraduate Medical School, London, I am indebted for his continued interest, advice and encouragement in the research on haemolytic anaemias in Iceland. I further express my gratitude to Professor Harry Harris and Dr Elizabeth B Robson at the M.R.C. unit of Human Biochemical Genetics, Galton Laboratory, London.

Of many Icelandic colleagues who have been helpful, I mention only Ingimar Hjálmarsson and Gísli Audunsson, Húusvík, Ólafur Sigurðsson and Magnús Ásmundsson, Akureyri.

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Last I cordially thank the numerous members of the families investigated, who have shown great understanding and co-operation.

from v W Ascertainment of asymptomatic v W family members with no or borderline changes in the coagulation bleeding tests would be important because such cases are among those who are prone to various hazards due to disease operations and drugs precipitating a bleeding episode By these recent immunological tests platelet aggregation test with ristocetin (Howard and

Firkin, 1971 Weiss et al 1972, *Ibidem*, 1973) and platelets adhesion tests to glass beads It has been possible to characterize genetic variants of v W (Holmberg and Nilsson (1972) and *ibidem* (1973) The riddle of v W is still to be solved and no single test or combination of tests are as yet available to ascertain the presence and type of gene or genes in every case

SUMMARY

Hereditary elliptocytosis

An Icelandic family with fifty elliptocytic individuals is reviewed. Pedigree studies indicate strongly that affected members of the family are descendants of a common ancestor The hereditary pattern is typical of a dominant autosomal gene with full penetrance A high incidence (over 57 per cent) of signs and/or symptoms of haemolysis is found among the affected at one time or another No haptoglobin was detected in 80 per cent of affected individuals investigated The age distribution of the affected at the time of diagnosis shows an increased incidence of anaemia with increasing age In three affected individuals splenectomy has been performed with satisfactory results The variation in the grades of haemolysis and the high incidence of haemolysis found in affected family members are discussed

Linkage analysis of 11 loci in 29 elliptocytic family units was done Close linkage of the elliptocytosis locus with the acid phosphate and Rhesus loci was excluded as was close linkage with other marker systems tested

Algerian-Icelandic family is reviewed in which the Algerian father had elliptocytosis as did one of his two children.

Hereditary spherocytosis (HS)

Thirty members with typical HS and over 70 apparently unaffected members belonging to 12 families have been studied. Splenec-

tomy has been performed on 22 HS patients Of nine HS individuals who had not undergone surgical treatment in 1857 four suffered from severe anaemia presumably due to aplastic crisis associated with influenza. One of them died a male 18 years of age

Pedigree studies on one of the families indicate that the HS gene or genes have been transmitted through six generations over the past 200 years

Marked deficiency in the number of affected compared with the apparently unaffected members in the HS families is present. The most striking example of the uneven genetic ratio is a sibship of 15 members investigated haematologically with one member suffering from typical HS

Much reduced penetrance of the HS gene or the presence of the so-called mild form is upheld as the main explanation for the unevenness in the genetic ratio Pedigree studies and haematological observations made are considered to support this However families are also present in which abortions and death at an early age indicated that selection against the affected could also disturb the genetic ratio in HS families

The Pelger anomaly

An Icelandic family containing fourteen members with Pelger anomaly is reviewed It is possible that this family is the only one with this type of mutation in Icelanders. No other Icelandic family has been found (see below) by scanning blood films from over 40000 Icelanders mainly in the Reykja-

vik area. Genealogical information indicates that the Pelger anomaly gene has been present in this family over 200 years.

The Pelger anomaly was diagnosed in 1974 in an American woman married to an Icelandic. One of their four children, a boy also has the anomaly.

Von Willebrand's disease

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APPENDIX

List of papers in English Danish and Icelandic by the author and his co-authors

In English

Jensson, Ó Observations on the Leucocyte Blood Picture in Acute Uraemia. Brit. J Haemat. 4, 422-427 1958.

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Supplementum 619

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FROM THE FIRST DEPARTMENT OF MEDICINE,
UNIVERSITY OF HELSINKI, AND FROM THE WIIHURI
RESEARCH INSTITUTE, HELSINKI, FINLAND

THE DIAGONAL EAR-LOBE CREASE,
A PHYSICAL SIGN ASSOCIATED WITH
CORONARY HEART DISEASE

BY
SIRKKA KAUKOLA

HELSINKI 1978

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INTRODUCTION

The incidence of coronary heart disease (CHD) has greatly increased throughout the world over the last 50 years up until recently CHD is the leading cause of death among males in western industrialized countries and the mortality among young and middle-aged men, particularly is high compared with that from other causes.

Coronary atherosclerosis is almost always responsible for CHD. Little is known of the rate of progression of coronary atherosclerosis, but it seems to be variable (Vlodaver and Edwards 1971 Bemis et al. 1973, Kumbhria et al. 1974). At least 2/4 of the lumen of a major coronary artery must be occluded before blood flow becomes significantly disturbed (Hood 1971). Even then, collateral circulation may protect the area distal to the occlusion against ischemia. On the other hand, 5 to 15 per cent of patients display ischemic symptoms in the absence of verifiable obstructive disease (Proudfit et al. 1964, Welch et al. 1970 and 1973, Friedberg 1972 and 1973, Day et al. 1977, Thompson et al. 1977).

Many epidemiological studies have revealed several risk factors which increase susceptibility to CHD and accelerate the progression of coronary atherosclerosis. However no causal relationship has been established between these factors and coronary atherosclerosis. The relative importance of these risk factors varies in different populations (Keys 1970 Dolder and Oliver 1974, Gertler

and White 1976), but three major ones — hypertension, hyperlipidemia and heavy cigarette smoking — are effectively becoming universally accepted. Data from different studies show that each of these can increase the risk of premature coronary artery disease independently of the others (Epstein and Ostrander 1971, Keys et al. 1972, Kannel et al. 1976), though divergent opinions have also been put forward (Werkö 1976). Age, sex, and heredity are important risk factors for ischemic heart disease in all populations. In addition, at least 40 minor risk factors have been described in the literature (Stamler et al. 1972, Fejfar 1973, Blackburn 1974), though their relative importance in CHD remains small or contradictory. Examples of minor risk factors are abnormal glucose tolerance, obesity sedentary life style and psychosocial factors, while still others are being found. Generally the impact of risk factors is viewed as additive.

As a disease of unknown pathogenesis and etiology coronary atherosclerosis remains an enigma. Some pathological evidence appears to favor the view that factors or changes related to the connective tissue of the arterial wall might play a role in the development of occlusive lesions (Cardinale and Udenfriend 1974 Osahima et al. 1975 and 1977, Fuller et al. 1976, Prockop et al. 1976). If so it is possible that the connective tissue disorder is universal and therefore likely to be detectable elsewhere in the body as well. Observations to support this are very scarce indeed, being

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limited to a few equivocal clinical observations.

Certain extracardiac signs have been considered to be associated with the presence of CHD or its clinical consequences. Myocardial infarction has been suggested to initiate the development of Dupuytren's contracture (Ask Upmark 1969) but its specificity as a sign of CHD is very low. During the 1970s it has become apparent that a diagonal ear-

lobe crease (EC) could be a sign of CE. Investigations to clarify this possible relationship and its specificity and sensitivity are still few in number however and the opinions thus generated have been contradictory.

The following literature survey summarizes the published evidence concerning the relationship between EC and ischemic heart disease.

SURVEY OF THE LITERATURE

Frank (1973) was first to describe a prominent crease in the lobule portion of the auricle and call it a positive ear-lobe sign. He studied 29 patients aged 60 years or less with this sign in terms of personal and family histories of premature cardiovascular disease and known risk factors. All but one displayed 1 or more of 6 possible risk factors, viz. hypercholesterolemia, hypertriglyceridemia, abnormal glucose tolerance, hypertension, cigarette smoking and a family history of premature cardiovascular disease. Two patients had angina and a third patient's electrocardiogram indicated ischemia. Frank suggested that the positive ear lobe sign was associated with premature cardiovascular disease.

The crease was later re-named the diagonal ear lobe crease (Lichstein et al. 1974) a term which has been in common use ever since. Lichstein et al. examined 631 patients with acute myocardial infarction over a three-year period. The age range was from 30 to 89 years, but no details of the patient selection were reported. Two hundred and fifty-one patients (47 %) exhibited EC, whereas it was present in only 33 (30 %) in 303 age-matched control patients with no clinical evidence of CHD. In the CHD group the prevalence of diabetes mellitus and hypertension was similar in those with and without EC. Smoking in patients with CHD and EC was significantly more common than in those with CHD but without EC, although no significant difference was found in the prevalence of

smoking in the control group with or without EC. These workers regarded EC as a coronary risk factor with an increasing prevalence between the sixth and ninth decades. They suggested that EC is not present at birth but develops later in life, the mechanism remaining to be clarified.

To determine the possible relationship between EC and CHD Sternlieb et al. (1974) undertook a study of 144 unselected patients in whom the presence or absence of coronary artery disease was verified by coronary arteriography. One hundred and twenty (90 %) of the 133 patients with EC had CHD whereas 10 of the 11 (91 %) without EC had normal coronary arteries.

In another study on 211 consecutive patients undergoing coronary arteriography the relationship of EC and CHD was examined (Mehta and Hamby 1974). One hundred and fifty-nine of the 211 patients had CHD while 52 had normal coronary arteries. No statistically significant difference was present between the frequency of EC in patients with CHD (58 %) and those with normal coronary arteries (60 %). The only correlation was between the frequency of EC and advancing age: it was present in 25 % of the patients below 55 years and in 65 % of those above.

Christiansen et al. (1975) agreed with Mehta and Hamby (1974) that the prevalence of EC increases with age. Their results also supported the findings of Lichstein et al. (1974) that the presence of EC was positively correlated with CHD. In this study (Christiansen

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OBJECTS OF THE PRESENT STUDY

The ear lobe crease (EC) is an easily detectable sign which might be useful in the diagnosis of CHD, although its clinical significance has not been established. The purpose of the present study was.

- to examine whether a statistical correlation exists between EC and CHD
- to determine whether there is a statistical relationship between EC and the major

and some minor coronary risk factors, viz. hypertension, hyperlipidemia, cigarette smoking, diabetes, obesity and sedentary living; also taking into account heredity and blood group, and factors such as other cardiovascular disease, birthplace and drug treatment (especially with beta-blockers).

- to evaluate the developmental features of EC.

et al.) a closer relationship was found between CHD and EC than between CHD and the risk factors of arterial hypertension, cigarette smoking and diabetes mellitus.

A significant positive correlation between EC and diabetic retinopathy was found by Andresen et al. (1976) who examined 101 diabetic patients. EC was seen in 51.4 % of the patients with retinopathy (mean age 53.9 ± 13.8 years) but in only 7.6 % of those with normal retinal vessels (mean age 52.7 ± 13.7 years). They considered this observation to indicate a correlation between EC and generalized angiopathy.

The data of Sprague (1976) suggest that intraoperative cardiovascular complications occur more frequently in patients with EC. The prevalence of postoperative complications was also greater in those with this sign. EC was regarded to be associated with CHD and to become more common with each decade above the third.

The frequency of EC and the extent and severity of coronary atherosclerosis have been compared in one postmortem study (Lichstein et al. 1976). The investigation involved 113 consecutive autopsies on patients aged 40 years or over comprising 32 patients without EC, 22 with unilateral EC, and 59 with bilateral EC. The extent of sclerosis of the coronary arteries was more severe in those with bilateral EC than in those without EC, the difference being statistically significant.

However it was not significantly different between those with unilateral EC and those without EC. The degree of occlusion did not differ significantly between the groups.

Doering et al. (1977) examined 50 patients with CHD of any type and 38 control patients in the age range 30 to 80. The groups were not age-matched, but 18 pairs of patients were matched with regard to age, sex, and CHD or control information. The prevalence of EC was 88.0 % in all CHD patients and 36.8 % in the controls, and in the age- and sex matched groups 94.4 % and 44.4 %, respectively.

Frank (1977) who was first to describe EC later found this sign in two men, aged 40 and 51 years, with classical angina and positive exercise tests but angiographically normal coronary arteries. Neither had hypertension nor diabetes.

Rhoads et al. (1977) examined 1237 Japanese men aged 50 to 74 from a cross section of the population in Hawaii and observed no relationship between CHD and EC which was present in 30 % of 71 men with CHD of any type, in 22 % of 37 men with myocardial infarction, and in 32 % of 116 men without CHD. EC was found rather to be weakly correlated with high blood pressure and strongly with obesity. They criticized the reliability of the detection of EC and also that obesity had not been taken into consideration in previous studies.

OBJECTS OF THE PRESENT STUDY

The ear-lobe crease (EC) is an easily detectable sign which might be useful in the diagnosis of CHD although its clinical significance has not been established. The purpose of the present study was.

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Frank (1977) who was first to describe EC later found this sign in two men, aged 53 and 51 years, with classical angina and positive exercise tests but angiographically normal coronary arteries. Neither had hypertension nor diabetes.

Rhoads et al. (1977) examined 1237 Japanese men aged 50 to 74 from a cross-section of the population in Hawaii and observed no relationship between CHD and EC which was present in 30 % of 71 men with CHD of any type in 22 % of 37 men with myocardial infarction, and in 32 % of 1166 men without CHD. EC was found rather to be weakly correlated with high blood pressure and strongly with obesity. They criticized the reliability of the detection of EC and also that obesity had not been taken into consideration in previous studies.

OBJECTS OF THE PRESENT STUDY

The ear lobe crease (EC) is an easily detectable sign which might be useful in the diagnosis of CHD, although its clinical significance has not been established. The purpose of the present study was:

- to examine whether a statistical correlation exists between EC and CHD
- to determine whether there is a statistical relationship between EC and the major

and some minor coronary risk factors, viz. hypertension, hyperlipidemia, cigarette smoking, diabetes, obesity and sedentary living also taking into account heredity and blood group and factors such as other cardiovascular disease, birthplace and drug treatment (especially with beta-blockers).

- to evaluate the developmental features of EC.

MATERIAL AND METHODS

The clinical study was made in two parts. In the first, patients with acute myocardial infarction were compared with an age- and sex matched control group. In the second part, patients with anginal symptoms who had been examined by coronary angiography were studied. They were divided into two groups: those with CHD and those with normal coronary arteries.

PATIENTS WITH ACUTE MYOCARDIAL INFARCTION (AMI)

The group comprised 219 patients surviving acute myocardial infarction (AMI) who were admitted to the First, Second, and Third Departments of Medicine Helsinki University Central Hospital, from March through May and from September through November 1976. There were 165 men aged 32 to 65 years and 54 women aged 43 to 65 with AMI (AMI patients, Table 1) fulfilling the criteria of

WHO (Working Group of Ischaemic Heart Disease Registers, EURO 8201 (3) 1971). Patients aged 65 years or over were excluded. During the period of collection of the patient material a total of 288 patients under 65 and with AMI were admitted to this hospital. Thus the patient sample involved about 76 % of all the survivors of AMI hospitalized and, although strictly neither a consecutive nor random patient sample it is probably fairly representative.

After admission, any history of chest pain was enquired about and serial electrocardiographic recordings and enzyme determinations were made as necessary. The serum enzyme most commonly determined was serum glutamine oxalacetic transaminase (S-ASAT) and, occasionally, serum creatine phosphokinase (S-CK). Serum levels were regarded as elevated if ASAT was 40 U/l or above and CK 80 U/l or above. According to the extent of heart injury myocardial infarctions were divided into subendocardial and transmural infarctions on the basis of WHO criteria. AMI could be the first or a recurrent event, but the same patient was not included more than once.

The patients were examined by the author for the presence or absence of EC and personal interview was used to obtain the following data: family history of premature cardiovascular disease and diabetes; birth place; father's and mother's birthplaces; previous angina pectoris and myoc

Table 1 Age and sex distributions of patients with acute myocardial infarction

Sex	Age in years				Total
	30-39	40-49	50-59	60-65	
Males	10	48	68	41	165
Females	—	11	4	19	34
Total	10	57	72	60	219

infarction, arterial hypertension, other cardiovascular disease, diabetes, smoking habits, physical activity at work and during leisure time, and previous drug treatment. Body weight and height were recorded. Fasting blood glucose and serum cholesterol and triglyceride values were determined about two months after the acute attack to obviate the changes known to occur in these variables during the acute period of myocardial infarction (Welin 1948, Blöck et al. 1957, Miettinen 1957, Dodds and Mills 1959, Sowton 1962, Nicolayson and Westlund 1963, Allison et al. 1969). Values from earlier records were used for these criteria if the patients did not return for examination after this myocardial infarction attack. Blood group had been determined on the wards in most cases.

CONTROL SUBJECTS

The control group consisted of 290 subjects (236 men and 54 women) working for the State Railways in the Helsinki area. Selection began by all the railway employees working as clerks, guards and switchmen in the Helsinki area being classified by sex, age and occupation. From each occupational group 100 subjects were drawn so that their age distribution corresponded to that of the AMI cases in the Helsinki area. The method of forming the control group has been described in detail earlier (Romo 1977).

The examination was carried out at the Railway Health Centre, Helsinki, during August and September 1976 as follows. Every subject received a questionnaire concerning the same data as the patients with AMI were asked about. The data in the completed forms were checked in a later interview. The presence or absence of EC was noted and the electrocardiogram, blood pressure, body weight and height were recorded. Determinations of fasting blood glucose, serum cholesterol and triglycerides

were made at the Department of Clinical Chemistry Helsinki University Central Hospital. After this the final control group was formed excluding the 10 subjects with previous myocardial infarction or abnormal Q-waves or ST-segment and T wave abnormalities in the resting electrocardiogram according to the criteria of the Minnesota code (Rose and Blackburn 1958).

In the final group men were distributed approximately equally as clerks, guards or switchmen, while all women were clerks. Table 2 gives their age distributions. The age

Table 2 Age and sex distributions of control subjects

Sex	Age in years				Total
	30-39	40-49	50-59	60-69	
Males	13	87	121	5	226
Females	—	18	24	16	54
Total	13	99	153	23	290

of men ranged from 22 to 63 years, but there were only 5 men over 59 years due to the early retirement age of railway employees in most jobs. The male control group was therefore well age-matched with the male infarction group only up to 59 years, the mean age of the younger than this being 50 years. Female subjects ranged from 43 to 63 years, with a mean of 57. The female control group was well age-matched with the female infarction patients.

PATIENTS STUDIED BY CORONARY ANGIOGRAPHY

Two hundred and eighty-six patients studied by coronary angiography at the Department of Diagnostic Radiology Helsinki University Central Hospital, made up this group. The

study was carried out from March, 1976 to April, 1977. One hundred and two consecutive patients were examined when they came to hospital for coronary angiography or coronary bypass surgery. The other 184 patients were invited for examination in March and April, 1977. These patients were living in the Helsinki area and were selected from those having had coronary angiography in 1971-76 and who were willing to cooperate. Thus the patient group as a whole should be considered as a selected one.

Coronary angiography had been performed on the patients because of angina pectoris or atypical angina pectoris or unexplained ischemic electrocardiographic changes in a bicycle ergometer test. The angiography was selective in 280 cases, while a semi-selective technique was used for 8 others. The findings were analyzed by two experienced radiologists. Otherwise, the same data were collected from these patients as mentioned earlier. In addition determinations of serum total lipids and lipoprotein electrophoresis on agarose gel were performed. Hyperlipoproteinemias were divided into subgroups based on WHO recommendation (Beaumont et al. 1970). Further attention was paid to serum alpha lipoprotein or high density lipoprotein (HDL) the level of which has been found to be low in patients with CHD (Nikkilä 1953, Lewis et al. 1974, Müller and Müller 1975, Berg et al. 1976).

The patients studied by coronary angiography were divided into subgroups according to the extent of coronary artery disease. Each individual coronary artery was evaluated by assessing the extent of coronary atherosclerosis present. Particular attention was focused on the right coronary artery (RCA), the left main coronary artery (LCA), the left anterior descending coronary artery (LAD) and the left circumflex coronary artery (LCA). If the degree of stenosis was 50 % or more in at least one of these coronary

Table 3 Age and sex distributions of CHD patients

Sex	Age in years						Total
	20-29	30-39	40-49	50-59	60-64	≥65	
Males	1	20	63	72	13	17	186
Females	—	7	10	8	1	5	31
Total	1	27	73	80	14	22	207

arteries, there was considered to be single, double, or triple vessel disease according to the number of arteries affected. Significant coronary atherosclerosis was found in 20 patients (CHD patients, Table 3) i.e. 50 with single vessel disease, 60 with double vessel disease and 84 with triple vessel disease. The other group consisted of 188 patients with no significant atherosclerotic changes in coronary arteries (non-CHD patients, Table 4). Some

Table 4 Age and sex distributions of non-CHD patients

Sex	Age in years						Total
	20-29	30-39	40-49	50-59	60-64	≥65	
Males	2	9	15	8	1	35	69
Females	—	8	34	17	2	31	92
Total	2	17	49	25	3	66	161

insignificant involvement (occlusion less than 50 %) was found in 11 cases and two investigations revealed a hypoplastic RCA, while 73 cases had fully normal coronary arteriogram.

The CHD group consisted of 174 men aged 26-66 and 26 women aged 30-66. The mean age was 48 years for both sexes. In the non-CHD group were 35 men and 51 women, the age range for men being from 6 to 60 years and for women from 32 to 66 years. The mean age was 51 years for both sexes.



Fig 1 A typical diagonal ear lobe crease in the lobule of the auricle

DEFINITIONS

The diagonal ear-lobe crease

The sign is clearly discernable as a downwards oblique crease in the lobule of the auricle (Fig 1).

In this study the patients were examined for the presence or absence of EC, and if present the sign was recorded as either bilateral or unilateral. Only a clear-cut crease was accepted as a positive finding. The examination was performed by the

author in all cases. To confirm the reliability of this investigation a material of 500 unselected subjects was examined by an experienced internist and by the author independently. The difference was 2.5 %.

Assessment of smoking habits

The patients and subjects were divided into smokers, ex-smokers and non-smokers as follows. a smoker was defined as a person who smoked at least one cigarette a day. Smokers were further divided into subgroups

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The patients studied by coronary angiography were divided into subgroups according to the extent of coronary artery disease. Each individual coronary artery was evaluated by assessing the extent of coronary atherosclerosis present. Particular attention was focused on the right coronary artery (RCA), the left main coronary artery (LCA), the left anterior descending coronary artery (LAD) and the left circumflex coronary artery (LCX). If the degree of stenosis was 50

Table 3 Age and sex distributions of CHD patients

Sex	Age in years					
	20-29	30-39	40-49	50-59	60-68	Total
Males	1	20	68	72	13	174
Females	—	7	10	8	1	26
Total	1	27	78	80	14	200

arteries, there was considered to be single, double, or triple vessel disease according to the number of arteries affected. Significant coronary atherosclerosis was found in 200 patients (CHD patients, Table 3), i.e. 50 with single vessel disease, 66 with double vessel disease and 84 with triple vessel disease. The other group consisted of 86 patients with no significant atherosclerotic changes in coronary arteries (non-CHD patients, Table 4). Some

Table 4 Age and sex distributions of non-CHD patients

Sex	Age in years					
	20-29	30-39	40-49	50-59	60-68	Total
Males	2	9	15	8	1	35
Females	—	8	24	17	2	51
Total	2	17	39	25	3	86

insignificant involvement (occlusion less than 50 %) was found in 11 cases and two investigations revealed a hypoplastic RCA while 73 cases had fully normal coronary arteriogram.

The CHD group consisted of 174 men aged 26-68 and 26 women aged 30-68. The mean age was 48 years for both sexes. In the non-CHD group were 35 men and 51 women, the age range for men being from 6 to 60 years and for women from 32 to 68 years. The mean age was 51 years for both sexes.



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The patients and subjects were divided into smokers, ex-smokers and non-smokers as follows: a smoker was defined as a person who smoked at least one cigarette a day. Smokers were further divided into subgroups

according to daily consumption of cigarettes. Pipe or cigar smokers constituted a group of their own. Ex smokers had given up smoking at least six months before the investigation. Non smokers had never smoked.

Assessment of obesity

Body weight and height were used to calculate the relative body weight using Quetlet's formula $\frac{\text{weight (kg)}}{\text{height (cm)}^2} \times 100$ (Khosla and Lowe 1987). A relative body weight of 0.28 was regarded as the limit for obesity

Assessment of physical activity

Physical activity both at work and during leisure time was enquired of the AMI patients and the control group. Attention was paid to the daily duration of sitting standing and walking and to the lifting and carrying of heavy objects when standing and walking.

Classification was accomplished according to the following definitions (Saltin and Grimby 1983, Wilhelmsen et al. 1971)

Activity at work

Group I (inactive) Mainly sitting

Group II (medium) Most of the time spent standing or walking. Rarely lifting or carrying heavy objects.

Group III (active) Much walking possibly also climbing stairs. Occasionally lifting or carrying heavy objects.

Group IV (very active) Frequent lifting or carrying heavy objects in addition to much walking

Leisure activity

Group I (inactive) No physical activity during leisure time.

Group II (medium) Walking, cycling, fishing

Group III (active) Regular sporting activity or heavy gardening or physical work at home for at least 2—3 hours weekly

Group IV (very active) Much sporting activity many times weekly

Birthplace

Due to the varying incidence of CHD in different regions of Finland (Keys 1970) the birthplace was taken into account. The inhabitants of western and eastern Finland may also differ genetically (Kajanoja 1971). However coronary atherosclerosis seems to develop to the same extent in individuals of both populations (Rissanen 1974). On the basis of these studies the country was divided into

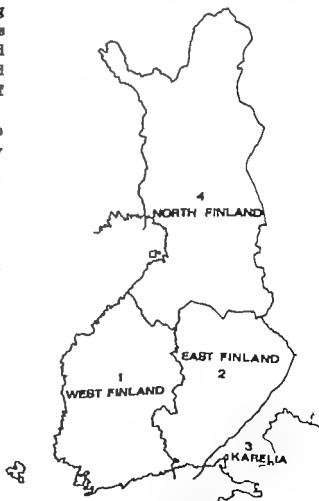


Fig. The country divided into three regions for coding the patients and subjects into groups by birthplace. The map also shows Karelia.

3 regions. — west Finland, east Finland, and
14 north Finland — and a fourth was former
Finnish Karelia — the area from which
8 people were resettled in other parts of Finland after World War II (Fig. 2).

STATISTICAL METHODS

Student's t-test and the chi square test were used in estimating the differences between the means.

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Assessment of physical activity

Physical activity both at work and during leisure time was enquired of the AMI patients and the control group. Attention was paid to the daily duration of sitting, standing and walking and to the lifting and carrying of heavy objects when standing and walking.

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- Group I (inactive) Mainly sitting
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- Group III (active) Much walking possibly also climbing stairs. Occasionally lifting or carrying heavy objects.
- Group IV (very active) Frequent lifting or carrying heavy objects in addition to much walking.

Leisure activity

- Group I (inactive) No physical activity during leisure time.
- Group II (medium) Walking, cycling, fishing, moderate gardening or physical work at home for at least 4 hours weekly.

- Group III (active) Regular sporting activity or heavy gardening or physical work at home for at least 2—3 hours weekly.
- Group IV (very active) Much sporting activity many times weekly.

Birthplace

Due to the varying incidence of CHD in different regions of Finland (Keys 1970), birthplace was taken into account. The inhabitants of western and eastern Finland may also differ genetically (Kajanoja 1971). However, coronary atherosclerosis seems to develop to the same extent in individuals from both populations (Rissanen 1974). On the basis of these studies the country was divided into



Fig. — The country divided into three regions for coding the patients and subjects into strata by birthplace. The map also shows Karelia — the easternmost area of Finland before World War II.

3 regions: — west Finland, east Finland, and north Finland — and a fourth was former Finnish Karelia — the area from which people were resettled in other parts of Finland after World War II (Fig 2).

STATISTICAL METHODS

Student's *t*-test and the chi-square test were used in estimating the differences between the means.

RESULTS

A. PATIENTS WITH MYOCARDIAL INFARCTION AND CONTROL SUBJECTS

PREVALENCE OF THE DIAGONAL EAR LOBE CREASE

Patients with myocardial infarction

EC was found in 151 of 219 AMI patients (69 %). The frequency was the same (69 %) in both men and women (Table 5). The prevalence of EC among the youngest AMI patients was remarkable — seven of the 10 male patients (70 %) aged 30—39 years had EC. The increasing prevalence with age was not statistically significant. The youngest man with EC was 32 years and the youngest woman 43. They were also the youngest AMI patients of either sex.

EC was more often bilateral than unilateral at all ages. However the unilateral EC was fairly common in men up to 49 years of age

In women it was more frequently unilateral than bilateral up to 59 years.

Control subjects

EC was present in 71 of 290 subjects (24 %). The sign was found in 66 of 236 men (28 %) while only 5 women (9 %) had it (Table 6). The prevalence did not show a statistically significant increase with age in these groups. Only one man under 40 had the sign, and in women it was not found in those under 50 years. The youngest man with EC was 35 years of age and the youngest woman 57 whereas the youngest man and woman in the whole control group were 32 and 43 respectively.

Both the unilateral and bilateral EC were found fairly equally among those subjects

Table 5 Percentage of AMI patients having the ear lobe crease by age and sex. Figures in parentheses indicate proportions of total groups

Sex	Age in years				Total
	30—39	40—49	50—59	60—69	
Males	9 (7.16)	61 (28.44)	71 (48.68)	6 (21.41)	69 (114.12)
Females	—	46 (5.11)	71 (17.24)	79 (15.19)	67 (37.54)
Total	9 (7.16)	58 (33.57)	71 (43.82)	77 (46.60)	69 (151)

Table II. Percentage of control subjects having the ear-lobe crease by age and sex. Figures in parentheses indicate proportions of total groups

Sex	Age in years				Total
	30-39	40-49	50-59	60-69	
Males	2 (1/13)	24 (21/83)	31 (41/131)	— (3/5)	28 (66/236)
Females	—	— (—/12)	12 (2/24)	11 (3/18)	9 (2/24)
Total	2 (1/13)	24 (21/89)	28 (64/184)	22 (5/23)	24 (71/298)

aged 40-49 years, but the bilateral EC was more common in men aged 50 or over. In women EC was seen so infrequently that comparison in this respect is impossible.

Patients with myocardial infarction compared to control subjects

The prevalence of EC was 69% in the AMI patients and 24% in the control subjects. The corresponding percentages were 66% and 25% in those below 60 years of age, where the groups were well age-matched and thus fully comparable. These differences were significant ($p < 0.01$) for the age group 30-39 years and highly significant ($p < 0.001$) for the other groups (Fig. 3).

PREVALENCE OF CORONARY RISK FACTORS IN PATIENTS WITH MYOCARDIAL INFARCTION AND CONTROL SUBJECTS

The AMI and control groups were adequately age-matched between 30 and 59 years of age and thus the prevalences of coronary risk factors in patients and controls were compared in this age range only. The former group comprised 169 patients and the latter 767 subjects.

History of arterial hypertension

A history of hypertension was revealed by 57 AMI patients (34%) 35% of men and

46% of women. The highest prevalence was found in the 40 to 49 year age group in both men (35%) and women (35%) but then the frequency decreased with age, being clearly higher in women than in men for all age groups.

In the control group hypertension had been previously diagnosed in 55 subjects (10%). The prevalence was the same for men and women. None of the women under 50 years had hypertension. However men had an

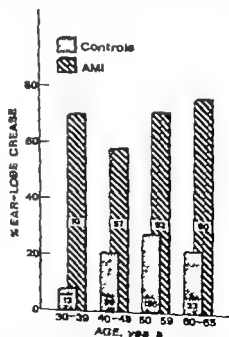


Fig. 3. Prevalence of the ear-lobe crease in AMI patients and controls by age. The numbers in the columns indicate the numbers of all individuals in each group.

RESULTS

A. PATIENTS WITH MYOCARDIAL INFARCTION AND CONTROL SUBJECTS

PREVALENCE OF THE DIAGONAL EAR LOBE CREASE

In women it was more frequently unilateral than bilateral up to 50 years.

Patients with myocardial infarction

EC was found in 151 of 219 AMI patients (69 %) The frequency was the same (69 %) in both men and women (Table 5) The prevalence of EC among the youngest AMI patients was remarkable, — seven of the 10 male patients (70 %) aged 30—39 years had EC The increasing prevalence with age was not statistically significant. The youngest man with EC was 32 years and the youngest woman 43 They were also the youngest AMI patients of either sex

EC was more often bilateral than unilateral at all ages However the unilateral EC was fairly common in men up to 49 years of age

Control subjects

EC was present in 71 of 230 subjects (24 %). The sign was found in 66 of 238 men (28 %) while only 5 women (9 %) had it (Table 6) The prevalence did not show a statistically significant increase with age in these groups Only one man under 40 had the sign, and in women it was not found in those under 50 years The youngest man with EC was 38 years of age and the youngest woman 57 whereas the youngest man and woman in the whole control group were 32 and 43 respectively

Both the unilateral and bilateral EC were found fairly equally among these subjects

Table 5 Percentage of AMI patients having the ear lobe crease by age and sex Figures in parentheses indicate proportions of total groups

Sex	Age in years				Total
	30—39	40—49	50—59	60—69	
Males	70 (7/10)	61 (22/46)	71 (48/68)	6 (3/41)	69 114 165
Females	—	46 (5/11)	71 (17/24)	79 (15/19)	69 87 144
Total	70 (7/10)	58 (33/57)	71 (65/92)	77 (18/50)	69 151 219

Table 2. Mean relative body weights in AMI patients and control subjects

x	AMI patients		Control subjects		p
	Mean	S.D.	Mean	S.D.	
Index	0.261	0.033	0.250	0.030	N.S.
Smokes	0.242	0.033	0.253	0.042	N.S.
Total	0.234	0.033	0.250	0.033	N.S.

valence being 54.5 % for men and 75.5 % for women. In the control group the corresponding percentages were 32 %, 30 % and 43 %. The differences were highly significant ($p < 0.001$).

The prevalence of history of hypertension in the families of AMI patients was 30 %, being again higher in women (38 %) than in men (28 %). 11 % of control subjects had hypertension in the family and the proportion was the same for both sexes. The difference between the groups was almost significant ($p < 0.05$).

With regard to history of diabetes in the family positive answers were given by 19 of AMI patients, 21 % of men and 18 % of women. The corresponding proportions were 25 %, 25 % and 28 % in the control group. In this case the differences were not significant.

The birthplace of the AMI patients and their parents was very often the same, and the situation was similar in the control group. There were no statistically significant differences between the groups here, either

THE DIAGONAL EAR LOBE CREASE AND CORONARY RISK FACTORS

The relationship between EC and coronary risk factors was studied in AMI patients and control subjects separately. Both groups were divided into those with EC, either bilaterally or unilaterally (EC patients and subjects) and those without EC (NEC patients and subjects).

Coronary risk factors in AMI patients with and without the ear lobe crease

The distribution of the AMI group as EC and NEC patients in different age groups is shown in Table 3.

History of arterial hypertension

The prevalence of hypertension was 34 % in the EC and 38 % in the NEC patients, which was not a statistically significant difference and neither were those for different age groups.

Serum lipids

Serum cholesterol

The mean serum cholesterol of the EC patients was 7.7 ± 1.5 mmol/l (mean \pm S.D.), whereas in the NEC patients it was 7.3 ± 1.4 mmol/l. The same trend was noted in different age groups except in those over 60 years (Fig. 4). The differences were statistically non-significant.

Table 3. AMI patients classified as those with (EC) or without (NEC) the ear-lobe crease by age. Figures in parentheses indicate percentages

Group	Age in years				Total
	30-39	40-49	50-59	60-69	
EC	7 (76)	23 (34)	65 (71)	46 (76)	151 (60)
NEC	3 (30)	24 (42)	27 (29)	14 (23)	68 (31)

Table 7 Mean serum cholesterol and triglyceride values in AMI patients and control subjects

Sex	AMI patients			Control subjects			P
	N	mmol/l	S.D.	N	mmol/l	S.D.	
Serum cholesterol							
Males	130	7.5	1.4	236	7.0	1.2	<0.01
Females	45	7.7	1.9	54	7.3	1.3	<0.01
Total	175	7.5	1.6	290	7.0	1.2	<0.01
Serum triglycerides							
Males	128	2.30	1.15	235	1.58	1.09	<0.001
Females	41	2.18	2.55	54	1.64	1.16	<0.001
Total	169	2.27	1.60	289	1.58	0.85	<0.001

equal prevalence of hypertension in all age groups over 40 years.

The difference in the prevalence of hypertension between the total patient and control groups was statistically highly significant ($p < 0.001$).

Serum lipids

Table 7 presents the mean serum cholesterol and triglyceride concentrations for both AMI and control subjects indicating also the significance of the differences between the groups.

Smoking

Among AMI patients 53 % were current smokers and 27 % ex smokers. Consumption of ≥ 20 cigarettes a day or more was the most common habit (34 %). All 10 patients under 40 years of age were currently smokers, 9 of them using more than 20 cigarettes a day. 38 % of men and 20 % of women consumed more than 20 cigarettes a day. Less than 20 cigarettes a day were smoked by 16.5 % of patients and cigars or a pipe by 3 %. 14 % of men and 39 % of women were non smokers.

In the control group there were 31 % current smokers, 39 % ex smokers and 31 % non-smokers. Only 4 % of women and 14 % of men smoked 20 cigarettes or more a day

while 20 % of women and 15 % of men smoked under 20 cigarettes a day. A pipe or cigars were smoked by only 2.4 % of this group. A considerable proportion of men (45 %) had given up smoking and most women (67 %) had never smoked.

The differences in smoking habits between the total patient and control groups were statistically highly significant ($p < 0.001$).

Diabetes mellitus

Diabetes had been previously diagnosed in 21.5 % of AMI patients and fasting blood glucose exceeded 5.3 mmol/l in a further 10 % of 205 patients (24 %). In the control group diabetes prevailed in only 4.5 % of subjects and fasting blood glucose was elevated in a further 8 of 231 subjects (3.5 %). The difference was statistically highly significant ($p < 0.001$).

Obesity

The mean relative body weights of the AMI and control groups are presented in Table 8.

Family history of premature cardiovascular disease and diabetes. Birthplace

Premature CHD was reported to have occurred in the family (i.e. parents, brothers and sisters) by 130 patients (60 %) the pre-

Table 8 Mean relative body weights of AMI patients and control subjects

Sex	AMI patients		Control subjects		P
	Mean	S.D.	Mean	S.D.	
Males	0.251	0.035	0.250	0.036	N.S.
Females	0.246	0.023	0.233	0.042	N.S.
Total	0.236	0.025	0.238	0.033	N.S.

valence being 54.5 % for men and 75.5 % for women. In the control group the corresponding percentages were 32 %, 30 % and 43 %. The differences were highly significant ($p < 0.001$).

The prevalence of history of hypertension in the families of AMI patients was 30 %, being again higher in women (36 %) than in men (23 %) 43 % of control subjects had hypertension in the family and the proportion was the same for both sexes. The difference between the groups was almost significant ($p < 0.05$).

With regard to history of diabetes in the family positive answers were given by 19 % of AMI patients, 21 % of men and 18 % of women. The corresponding proportions were 25 %, 25 % and 25 % in the control group. In this case the differences were not significant.

The birthplace of the AMI patients and their parents was very often the same, and the situation was similar in the control group. There were no statistically significant differences between the groups here, either

THE DIAGONAL EAR LOBE CREASE AND CORONARY RISK FACTORS

The relationship between EC and coronary risk factors was studied in AMI patients and control subjects separately. Both groups were divided into those with EC either bilaterally or unilaterally (EC patients and subjects) and those without EC (NEC patients and subjects).

Coronary risk factors in AMI patients with and without the ear-lobe crease

The distribution of the AMI group as EC and NEC patients in different age groups is shown in Table 9.

History of arterial hypertension

The prevalence of hypertension was 34 % in the EC and 38 % in the NEC patients, which was not a statistically significant difference and neither were those for different age groups.

Serum lipids

Serum cholesterol

The mean serum cholesterol of the EC patients was 7.7 ± 1.6 mmol/l (mean \pm S.D.), whereas in the NEC patients it was 7.2 ± 1.4 mmol/l. The same trend was noted in different age groups except in those over 60 years (Fig. 4). The differences were statistically non-significant.

Table 9 AMI patients classified as those with (EC) or without (NEC) the ear-lobe crease by age. Figures in parentheses indicate percentages

Group	Age in years				Total
	20-39	40-49	50-59	60-69	
EC	7 (70)	23 (34)	63 (71)	46 (76)	140 (60)
NEC	3 (30)	4 (6)	17 (19)	11 (18)	35 (15)

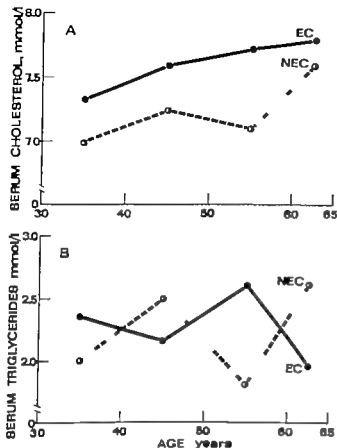


Fig. 4 Mean serum cholesterol (A) and triglycerides (B) of EC and NEC patients in the AMI group.

Serum triglycerides

Mean serum triglycerides were above the normal range in all age groups of both EC and NEC patients (Fig 4). The mean of the EC patients was 2.32 ± 1.78 mmol/l and that of the NEC patients 2.18 ± 1.91 mmol/l. The differences were not statistically significant.

Smoking

The EC patients were less frequently current smokers (52 %) and ex smokers (25 %) than

the NEC patients (58 % and 29 %). Non-smokers represented 23 % of the EC patients and 15 % of the NEC patients. The differences in daily consumption of cigarettes between these groups were small (Table 10), and none were statistically significant.

Diabetes mellitus

The prevalence of diabetes was 22 % in the EC and 21 % in the NEC patients (p NS).

Obesity

The mean relative body weight was 0.254 ± 0.037 in the EC and 0.257 ± 0.028 in the NEC patients. This difference was not statistically significant and neither were the means in different age groups (Fig 5).

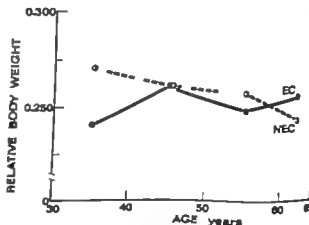


Fig. 5 Mean relative body weight of EC and NEC patients in the AMI group.

Level of physical activity

Fig 6 presents the distribution of the EC and NEC patients according to physical activity

Table 10 Distribution of EC and NEC patients in the AMI group by smoking habit. Figures in parentheses indicate percentages

Group	Current cigarette smoking		Cigarette	Ex smokers		Non-smokers	Total
	< 10 per day	≥ 10 per day					
EC	28 (17)	49 (33)	3 (2)	38 (25)	34 (23)		150
NEC	10 (15)	43 (37)	3 (4)	20 (29)	18 (15)		68

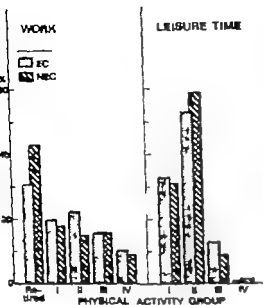


Fig. 6 Distribution of EC and NEC patients in the AMI group according to level of the physical activity at work and during leisure time

at work. They were dispersed fairly similarly throughout all four activity groups (p NS). 31 % of the EC patients and 43 % of the NEC patients were retired.

The physical activity during leisure time did not differ significantly between the EC and NEC groups. The distribution into four leisure activity level groups is shown in Fig. 6.

Blood group

The EC and NEC patients were distributed fairly similarly among the different blood groups: A 52 % and 54 %, B 16 % and 19 %, AB 11 % and 4 %, O 20 % and 23 %, respectively (p NS).

Family history of premature cardiovascular disease and diabetes. Birthplace

Premature CHD in the family was reported by 59 % of the EC and 51 % of the NEC patients (p NS).

A history of hypertension was considerably more frequent in the families of the NEC patients (43 %) than in those of the EC patients (24 %). This difference was statistically significant ($p < 0.01$).

Diabetes was equally represented in family histories of the EC (19 %) and NEC patients (18.5 %), without a statistically significant difference.

A relatively greater proportion of the NEC patients (58 %) and their parents (54 %) were born in eastern Finland compared to the EC patients (50 %) and their parents (48 % and 46 %). The situation was opposite for western Finland. With regard to Karelia the percentages were very similar in both groups (Fig. 7). None of the differences was statistically significant.

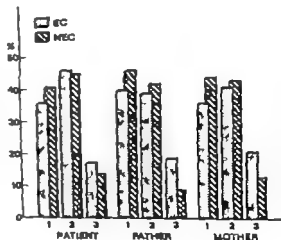


Fig. 7 Distribution of EC and NEC patients and their parents in the AMI group according to birthplace (1. west Finland, 2. east Finland, 3. Karelia).

Other characteristics of AMI patients with and without the ear-lobe crease

Previous and current history of CHD

The duration of angina pectoris had been fairly similar (p NS) in both groups. The EC patients had experienced symptoms for 2.3 ± 3.7 years and the NEC patients for 2.6 ± 3.5 years prior to this study.

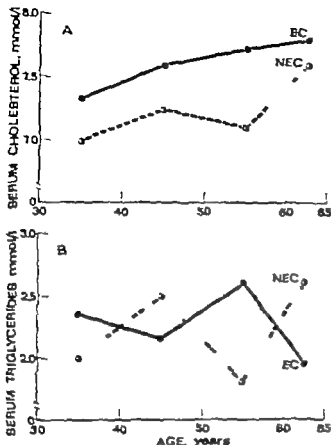


Fig 4 Mean serum cholesterol (A) and triglycerides (B) of EC and NEC patients in the AMI group.

Serum triglycerides

Mean serum triglycerides were above the normal range in all age groups of both EC and NEC patients (Fig 4). The mean of the EC patients was 2.2 ± 1.8 mmol/L and that of the NEC patients 2.18 ± 1.21 mmol/L. The differences were not statistically significant.

Smoking

The EC patients were less frequently current smokers (52%) and ex smokers (25%) than

the NEC patients (56% and 29%). Non-smokers represented 33% of the EC patients and 15% of the NEC patients. The differences in daily consumption of cigarettes between these groups were small (Table 10) and none were statistically significant.

Diabetes mellitus

The prevalence of diabetes was 1% in the EC and 21% in the NEC patients ($p < 0.05$).

Obesity

The mean relative body weight was 0.54 ± 0.037 in the EC and 0.257 ± 0.03 in the NEC patients. This difference was not statistically significant and neither were the means in different age groups (Fig 5).

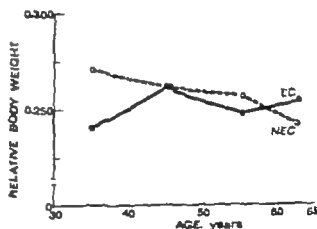


Fig 5 Mean relative body weight of EC and NEC patients in the AMI group

Level of physical activity

Fig 6 presents the distribution of the EC and NEC patients according to physical activity

Table 10 Distribution of EC and NEC patients in the AMI group by smoking habits. Figures in parentheses indicate percentages

Group	Current cigarette smoking		Cigarette	Ex smokers	Non smokers	Total
	< 10 per day	≥ 20 per day				
EC	6 (17)	49 (33)	3 (2)	28 (5)	34 (—)	50
NEC	10 (15)	35 (27)	3 (4)	3 (—)	10 (1)	51

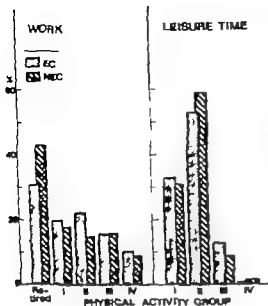


Fig. 6 Distribution of EC and NEC patients in the AMI group according to level of the physical activity: work and during leisure time

at work. They were dispersed fairly similarly throughout all four activity groups (p NS). 31% of the EC patients and 43% of the NEC patients were retired.

The physical activity during leisure time did not differ significantly between the EC and NEC groups. The distribution into four leisure activity level groups is shown in Fig. 6.

Blood group

The EC and NEC patients were distributed fairly similarly among the different blood groups: A 52% and 54%, B 16% and 19%, AB 11% and 4%, O 20% and 23%, respectively (p NS).

Family history of premature cardiovascular disease and diabetes. Birthplace

Premature CHD in the family was reported by 59% of the EC and 61% of the NEC patients (p NS).

A history of hypertension was considerably more frequent in the families of the NEC patients (43%) than in those of the EC patients (24%). This difference was statistically significant ($p < 0.01$).

Diabetes was equally represented in family histories of the EC (19%) and NEC patients (18.5%), without a statistically significant difference.

A relatively greater proportion of the NEC patients (58%) and their parents (54%) were born in eastern Finland compared to the EC patients (50%) and their parents (48% and 46%). The situation was opposite for western Finland. With regard to Karelia the percentages were very similar in both groups (Fig. 7). None of the differences was statistically significant.

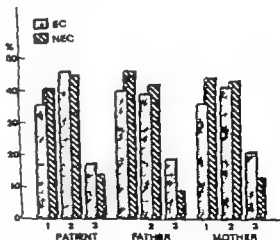


Fig. 7 Distribution of EC and NEC patients and their parents in the AMI group according to birthplace (1 west Finland, 2 east Finland, 3 Karelia).

Other characteristics of AMI patients with and without the ear-lobe crease

Previous and current history of CHD

The duration of angina pectoris had been fairly similar (p NS) in both groups. The EC patients had experienced symptoms for 2.3 ± 3.7 years and the NEC patients for 2.8 ± 2.5 years prior to this study.

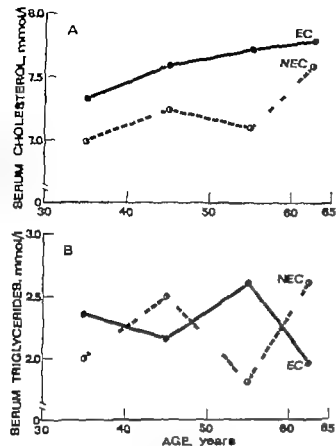


Fig 4 Mean serum cholesterol (A) and triglycerides (B) of EC and NEC patients in the AMI group.

Serum triglycerides

Mean serum triglycerides were above the normal range in all age groups of both EC and NEC patients (Fig 4). The mean of the EC patients was 2.32 ± 1.78 mmol/L and that of the NEC patients 2.18 ± 1.21 mmol/L. The differences were not statistically significant.

Smoking

The EC patients were less frequently current smokers (52 %) and ex-smokers (25 %) than

the NEC patients (56 % and 29 %). Non smokers represented 23 % of the EC patients and 15 % of the NEC patients. The differences in daily consumption of cigarettes between these groups were small (Table 10), and none were statistically significant.

Diabetes mellitus

The prevalence of diabetes was 22 % in the EC and 21 % in the NEC patients (p NS).

Obesity

The mean relative body weight was 0.254 ± 0.037 in the EC and 0.257 ± 0.028 in the NEC patients. This difference was not statistically significant and neither were the means in different age groups (Fig 5).

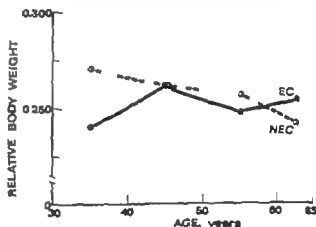


Fig 5 Mean relative body weight of EC and NEC patients in the AMI group.

Level of physical activity

Fig 6 presents the distribution of the EC and NEC patients according to physical activity,

Table 10 Distribution of EC and NEC patients in the AMI group by smoking habit. Figures in parentheses indicate percentages

Group	Current cigarette smoking		Cigar/pipe	Ex smokers	Non-smokers	Total
	< 10 per day	≥ 10 per day				
EC	26 (17)	49 (33)	3 (2)	38 (25)	34 (23)	150
NEC	10 (15)	25 (37)	3 (4)	20 (29)	10 (15)	68

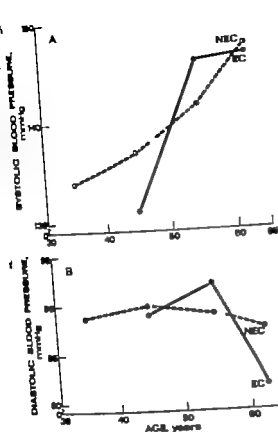


Fig 8 Mean systolic (A) and diastolic (B) blood pressure of EC and NEC controls.

Diastolic blood pressure

The mean diastolic blood pressure differed only slightly between the EC and NEC groups. It was 90 ± 10 mmHg in the former and 89 ± 11 mmHg in the latter. The differences were also small in the different age groups (Fig 8) without any statistical significance.

Serum lipids

Serum cholesterol

The mean serum cholesterol did not deviate significantly between the EC and NEC control subjects. The means were 7.0 ± 1.2 mmol/l and 7.1 ± 1.2 mmol/l, and those by age groups are indicated in Fig 9.

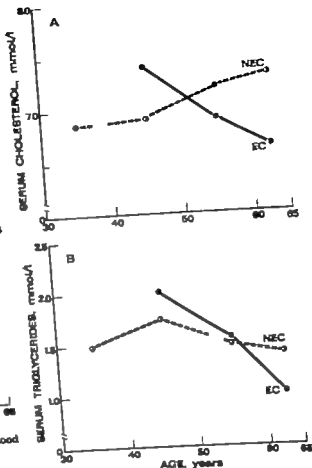


Fig 9 Mean serum cholesterol (A) and triglycerides (B) of EC and NEC controls.

Serum triglycerides

No significant difference could be found with respect to serum triglycerides in this comparison either. The means were 1.62 ± 1.24 mmol/l for the EC subjects and 1.58 ± 1.04 mmol/l for the NEC subjects, and the means by age groups were quite close to these (Fig. 9).

Smoking

Among controls the EC subjects were less frequently current smokers (22.5%) and non-smokers (28%) than the NEC subjects (32%).

The NEC patients seemed to have a trend of more previous myocardial infarctions (38 %) than the EC patients (29 %) though this difference was not statistically significant.

The present myocardial infarction was transmural in 71 % of the EC patients and 83 % of the NEC patients, and non-transmural in 29 % of the former group and 37 % of the latter. Again, none of these differences was statistically significant.

Other cardiovascular disease

Other cardiovascular disease had previously been diagnosed in 29 AMI patients (13 %) of whom 17 were men (10 %) and 12 women (22 %). Intermittent claudication was the most common (17 patients). Other cardiovascular diseases involved were cerebrovascular disease (5), stenosis of the neck arteries (3), atrial septal defect (1), aortic valvular insufficiency (1), operated ductus Botalli (1), and syndrome Raynaud (1). The distribution of these diseases was without any statistically significant difference between the EC (14 %) and NEC (18 %) patients.

Drug treatment

The AMI patients were questioned about their use of drugs, especially of beta-blockers and serum lipid lowering agents (clofibrate exclusively among AMI patients). Beta blockers were used by 68 patients (27 %) equally by both men and women, and

clofibrate by 9 patients (4.1 %). No significant differences were found with respect to the use of these drugs between the EC (2 % and 4 %) and NEC (31 % and 4 %) patients.

Coronary risk factors in control subjects with and without the ear lobe crease

In the control group there were 71 subjects (24 %) with EC and 219 subjects (76 %) without it. The prevalence of this sign was thus the ratio of the EC subjects to the EC ones (distribution by age groups shown in Table 11) were low compared with AMI patients. Otherwise, the following comparisons are in agreement with the corresponding ones in the AMI group.

Blood pressure

History of arterial hypertension

Hypertension had been previously diagnosed more frequently in the NEC (20 %) than in EC control subjects (15.5 %) but this was not a statistically significant difference.

Systolic blood pressure

The mean systolic blood pressure was equal in the EC (141 ± 18.5 mmHg) and NEC subjects (140 ± 19 mmHg) without a statistically significant difference. Differences were also non significant when calculated by age groups (Fig. 8).

Table 11 Control subjects classified as those with (EC) or without (NEC) the ear lobe crease by age. Figures in parentheses indicate percentages

Group	Age in years				Total
	30—39	40—49	50—59	60—69	
EC	1 (8)	21 (21)	44 (28)	5 (22)	71 (24)
NEC	13 (92)	78 (79)	111 (72)	18 (78)	219 (76)

distributed equally among different activity groups (Fig. 11), so that there were no statistically significant differences here, either.

Family history of premature cardiovascular disease and diabetes. Birthplace

Premature CHD was reported in the families of 30 of the EC and 33 of the NEC subjects. This small difference was not statistically significant.

Hypertension in the family was also more common in the EC (51 %) than in the NEC subjects (40 %) but the difference was statistically non-significant.

The family history for diabetes was positive in 29 of the EC and in 24 of the NEC subjects. This difference was not also statistically significant.

The proportional distributions of the EC and NEC subjects and their parents according to birthplace (Fig. 12) corresponded closely

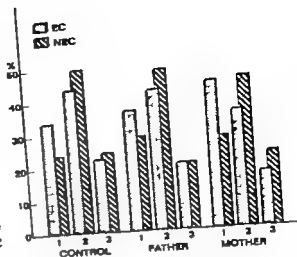


Fig. 12 Distribution of EC and NEC controls and their parents according to birthplace (1. west Finland, 2. east Finland, 3. Karelia).

to those of the AMI group. The differences were small in all respects and statistically non-significant.

Table 12 Distribution of EC and NEC controls by smoking habits. Figures in parentheses indicate percentages

Group	Current cigarette smoking		Cigar/pipe	Ex-smokers	Non smokers	Total
	< 20 per day	≥ 20 per day				
EC	6 (8.5)	10 (14)	—	33 (49)	20 (28)	71
NEC	39 (18)	25 (11)	7 (3)	78 (36)	11 (32)	219

but more frequently ex-smokers (49 %) than the latter (36 %). Twenty cigarettes or more a day were smoked in both groups quite equally (Table 12) but smokers at lower levels were more frequent among NEC (18 %) than EC subjects (8.5 %). The differences were not statistically significant.

Diabetes mellitus

The prevalence of diabetes was 7 % in the EC and 4 % in the NEC subjects, which was a statistically non significant difference.

Obesity

The mean relative body weight was 0.261 ± 0.030 in the EC and 0.256 ± 0.033 in the NEC subjects. This difference was statistically non significant as were the differences in the age groups over 40 years (Fig 10).

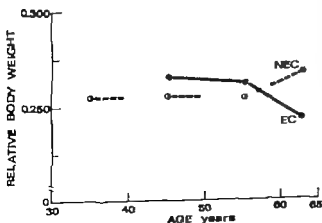


Fig 10 Mean relative body weight of EC and NEC controls.

Level of physical activity

The greatest differences were in the inactive (I) and medium (II) physical activity groups at work (Fig 11). The NEC subjects were more inactive (43 %) whereas the EC subjects performed more light exercise (35 %). However the differences were not statistically significant.

With regard to the physical activity during leisure time the EC and NEC subjects were

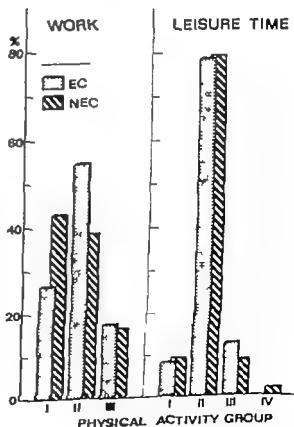


Fig 11 Distribution of EC and NEC controls according to level of the physical activity at work and during leisure time.

distributed equally among different activity groups (Fig. 11), so that there were no statistically significant differences here, either

Family history of premature cardiovascular disease and diabetes. Birthplace

Premature CHD was reported in the families of 30 of the EC and 33 of the NEC subjects. This small difference was not statistically significant.

Hypertension in the family was also more common in the EC (31) than in the NEC subjects (40), but the difference was statistically non-significant.

The family history for diabetes was positive in 29 of the EC and in 24 of the NEC subjects. This difference was not also statistically significant.

The proportional distributions of the EC and NEC subjects and their parents according to birthplace (Fig. 12) corresponded closely

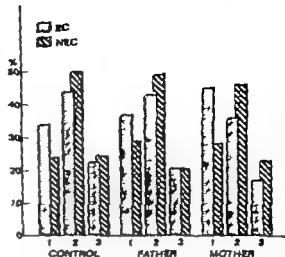


Fig. 12 Distribution of EC and NEC controls and their parents according to birthplace (1 west Finland, 2 east Finland, 3 Karelia).

to those of the AMI group. The differences were small in all respects and statistically non-significant.

B PATIENTS STUDIED BY CORONARY ANGIOGRAPHY

PREVALENCE OF THE DIAGONAL EAR LOBE CREASE

CHD patients

EC was present in 144 of 200 CHD patients (72 %) whereas it was absent in 56 patients (28 %). In this group there was only one patient under 30 years, and he had EC. Other wise the presence of EC was rather in frequent in patients below 40 years. In the age group 70 to 79 years it was seen in 10 of 27 patients (37 %). In the following decade

EC was found in as many as 51 of 78 patients (65 %) and above 80 years the prevalence increased still further to 70 of 80 patients (87 %) aged 50 to 59 and 12 of 14 patients (86 %) aged 60 to 69 (Table 13). This increase with age was not, however statistically significant.

Below the age of 40 years EC was present more frequently unilaterally whereas above 40 years of age the bilateral EC became dominant, and this trend further increased with age.

Table 13. Presence and absence of the ear-lobe crease in CHD patients by age. Figures in parentheses indicate percentages.

Ear lobe crease	Age in years					Total
	20—29	30—39	40—49	50—59	60—69	
Bilateral	—	3 (11)	35 (45)	53 (66)	10 (72)	101 (50.5)
Unilateral	1	7 (26)	18 (20)	17 (21)	2 (14)	43 (21.5)
Total	1	10 (37)	51 (65)	70 (87)	12 (86)	144 (72)
Absence	—	17 (63)	27 (35)	10 (12)	2 (14)	56 (28)

Table 14. Presence and absence of the ear lobe crease in non-CHD patients by age. Figures in parentheses indicate percentages.

Ear lobe crease	Age in years					Total
	20—29	30—39	40—49	50—59	60—69	
Bilateral	—	1 (6)	4 (10)	5 (20)	2	12 (14)
Unilateral	—	—	4 (10)	2 (8)	—	6 (7)
Total	—	1 (6)	8 (20.5)	7 (28)	2	18 (21)
Absence	2	15 (94)	31 (79.5)	18 (72)	1	68 (79)

Non-CHD patients

In the whole non-CHD group there were 68 of 86 patients (79 %) without EC and 18 (21 %) with it. Neither of the two patients under 30 years had EC, and in the 30 to 39 age group only one of 17 patients (6 %) had it (Table 14). Over 40 years of age the prevalence increased so that at the ages of 40 to 49 years EC was present in 8 of 39 patients (20.5 %) and at the ages of 50 to 59 years in 7 of 23 patients (30.4 %), although the increasing prevalence with age was not statistically significant in this group either. Two of the 3 patients over 60 years had EC.

Comparison of the unilateral and bilateral EC was not feasible in this group because of their low prevalences.

CHD patients compared to non-CHD patients

EC was found in 72 % of the CHD group and in 21 % of the non-CHD group, this difference being statistically highly significant ($p < 0.001$).

Most patients studied angiographically were between 30 and 59 years of age. During this period the prevalence of EC rose to each

decade in both groups, but the difference remained significant and even tended to increase with age (Fig. 13). Taking into account all patients under 60 years of age EC was found in 71 % of the CHD group and in 19 % of the non-CHD group.

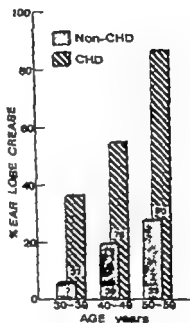


Fig. 13. Prevalence of the ear lobe crease in the largest age groups of CHD and non-CHD patients. The numbers in the columns indicate the numbers of all individuals in each group.

Table 15. Presence (EC) and absence (NEC) of the ear lobe crease in CHD patients by age and extent of coronary atherosclerosis. Figures in parentheses indicate percentages

Group	Age in years					Total
	20-29	30-39	40-49	50-59	60-64	
1-vessel disease						
EC	—	4 (29)	13 (38)	7 (84)	2	26 (32)
NEC	—	10 (71)	9 (41)	4 (36)	1	24 (48)
2-vessel disease						
EC	1	3 (43)	18 (80)	28 (93)	2	52 (79)
NEC	—	4 (57)	8 (31)	2 (7)	—	14 (21)
3-vessel disease						
EC	—	3 (30)	20 (87)	35 (90)	8 (89)	66 (79)
NEC	—	3 (30)	10 (33)	4 (10)	1 (11)	18 (21)

B PATIENTS STUDIED BY CORONARY ANGIOGRAPHY

PREVALENCE OF THE DIAGONAL EAR LOBE CREASE

CHD patients

EC was present in 144 of 200 CHD patients (72 %) whereas it was absent in 56 patients (28 %). In this group there was only one patient under 30 years, and he had EC. Otherwise the presence of EC was rather infrequent in patients below 40 years. In the age group 30 to 39 years it was seen in 10 of 27 patients (37 %). In the following decade

EC was found in as many as 51 of 78 patients (65 %) and above 50 years the prevalence increased still further to 70 of 80 patients (87 %) aged 50 to 59 and 12 of 14 patients (86 %) aged 60 to 66 (Table 13). This increase with age was not, however statistically significant.

Below the age of 40 years EC was present more frequently unilaterally whereas above 40 years of age the bilateral EC became dominant, and this trend further increased with age.

Table 13 Presence and absence of the ear lobe crease in CHD patients by age. Figures in parentheses indicate percentages

Ear lobe crease	Age in years					Total
	20—29	30—39	40—49	50—59	60—66	
Bilateral	—	3 (11)	35 (46)	53 (66)	10 (73)	101 (50.5)
Unilateral	1	7 (26)	16 (20)	17 (21)	3 (14)	44 (21.5)
Total	1	10 (37)	51 (65)	70 (87)	13 (86)	144 (72)
Absence	—	17 (63)	27 (35)	10 (12)	2 (14)	56 (28)

Table 14 Presence and absence of the ear-lobe crease in non-CHD patients by age. Figures in parentheses indicate percentages

Ear lobe crease	Age in years					Total
	20—29	30—39	40—49	50—59	60—69	
Bilateral	—	1 (6)	4 (10)	5 (20)	2	12 (14)
Unilateral	—	—	4 (10)	2 (8)	—	6 (7)
Total	—	1 (6)	8 (20.5)	7 (28)	2	18 (21)
Absence	2	16 (94)	31 (79.5)	16 (72)	1	66 (79)

Systolic blood pressure

In the CHD group the mean systolic blood pressure was higher in all age groups than in the non-CHD group. For the whole groups the means were 137 ± 17 mmHg and 133 ± 8 mmHg, respectively ($p < 0.01$)

Diastolic blood pressure

The relation between the groups was similar for the diastolic blood pressure. The means were higher in the CHD than the non-CHD group. In the former group the mean was 88 ± 10 mmHg and in the latter 84 ± 9 mmHg. The difference was statistically significant ($p < 0.001$)

Serum lipids

Serum cholesterol

The mean serum cholesterol concentrations in the CHD and non-CHD groups are shown in Table 16. In different age groups the mean values were also significantly higher in the CHD group compared to the non-CHD group except between those aged 50 to 59 years.

Serum triglycerides

The mean serum triglyceride concentrations were elevated in all age groups of the CHD patients, but normal in all age groups of the non-CHD patients. The total means of the groups are presented in Table 16

Table 17 Distribution of CHD and non-CHD patients according to type of hyperlipoproteinemia. Figures in parentheses indicate percentages

Group	No of patients	Type of hyperlipoproteinemia			
		0	II A	II B	IV
CHD	112	28 (34)	28 (33)	30 (27)	7 (6)
non-CHD	71	38 (53.5)	37 (39)	4 (6)	1 (1.4)

Serum total lipids

A similar ratio between the groups was apparent for serum total lipids as well (Table 16). The means rose with age in the same proportions in both CHD and non-CHD patients, so that similar differences were seen at each age group

Hyperlipoproteinemias

About a third of the CHD patients, but over a half of the non-CHD patients, had normal lipid values which excluded any type of hyperlipoproteinemia (Table 17). The differences between the groups were most obvious for type II B ($p < 0.001$) whereas for types II A and IV they were small (p NS).

Serum alpha lipoprotein

The levels of serum alpha-lipoprotein were lower in all age groups of CHD patients compared to non-CHD patients, as also were the total group means (Table 16).

Table 16 Mean serum lipids CHD and non-CHD patients. The differences between the groups were highly significant ($p < 0.001$)

Group	Cholesterol			Triglycerides			Total lipids			Alpha-lipoprotein		
	N	mmol/l	S.D.	N	mmol/l	S.D.	N	g/l	S.D.	N	g/l	S.D.
CHD	102	7.8	1.6	102	2.13	1.87	112	8.5	2.1	112	1.50	0.43
non-CHD	62	7.0	1.7	61	1.24	1.01	71	7.4	1.8	71	1.93	0.55

THE RELATIONSHIP OF THE EAR LOBE CREASE TO THE EXTENT OF CORONARY ATHEROSCLEROSIS

Single vessel disease

EC was seen in 28 of the 50 patients (52 %) with single vessel disease (Table 15) The prevalence was lowest at the ages of 30 to 39 in which group EC was found in only 4 of 14 patients (29 %) but the frequency tended to increase with age (Fig 14) At the ages of 40 to 49 EC was found in 13 of 22 patients (59 %) and in the 50 to 59 age group in 7 of 11 (64 %) Two of the 3 patients over 60 years had EC.

Double vessel disease

The prevalence of EC also tended to increase with age in patients with double vessel disease (Fig 14) The prevalence was higher

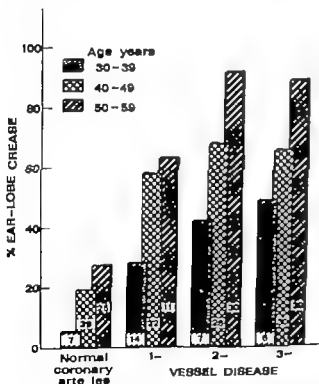


Fig 14 Prevalence of the ear lobe crease in patients studied by coronary angiography by age and extent of coronary atherosclerosis. The numbers in the columns indicate the numbers of all individuals in each group.

though not significantly in all age groups compared to those with single vessel disease EC was found in 52 of 66 patients (79 %) with double vessel disease. By age groups, it was present in 3 of 7 patients (43 %) at ages of 30 to 39 in 18 of 26 (69 %) at 40 to 49 and in 28 of 30 (93 %) in the 50 to 59 age group Only one patient was below 30 years and 2 were over 60 all three had EC (Table 15)

Triple vessel disease

In triple vessel disease the prevalence of EC was fairly similar to that in double vessel disease. In the whole group 66 of 84 patients (79 %) had EC At the ages of 30 to 39 it was seen in 3 of 6 patients (50 %) at 40 to 49 years in 20 of 30 patients (67 %) and at 50 to 59 years in 35 of 39 patients (90 %) The group also included 9 patients over 60 years of whom 8 (89 %) had EC (Table 15 Fig 14) The prevalence of EC did not differ statistically significantly from that in single vessel disease

PREVALENCE OF CORONARY RISK FACTORS IN CHD AND NON CHD PATIENTS

The following risk factors are described for the CHD and non-CHD groups, men and women combined. In addition, the comparisons have been done between the men of both groups too but this is mentioned only if the two sets of results differed from each other

Blood pressure

History of arterial hypertension

Hypertension had been diagnosed before this study in 62 CHD patients (31 %) and in 15 patients of the non-CHD group (17 %) The difference was significant ($p < 0.01$)

Systolic blood pressure

In the CHD group the mean systolic blood pressure was higher in all age groups than in the non-CHD group. For the whole groups the means were 137 ± 17 mmHg and 133 ± 16 mmHg, respectively ($p < 0.01$)

Diastolic blood pressure

The relation between the groups was similar for the diastolic blood pressure. The means were higher in the CHD than the non-CHD group. In the former group the mean was 88 ± 10 mmHg and in the latter 84 ± 9 mmHg. The difference was statistically significant ($p < 0.001$)

Serum lipids

Serum cholesterol

The mean serum cholesterol concentrations in the CHD and non-CHD groups are shown in Table 16. In different age groups the mean values were also significantly higher in the CHD group compared to the non-CHD group except between those aged 50 to 59 years.

Serum triglycerides

The mean serum triglyceride concentrations were elevated in all age groups of the CHD patients, but normal in all age groups of the non-CHD patients. The total means of the groups are presented in Table 16.

Table 17 Distribution of CHD and non-CHD patients according to type of hyperlipoproteinemia. Figures in parentheses indicate percentages

Group	No. of pts.	Type of hyperlipoproteinemia			
		0	II A	II B	IV
CHD	112	26 (24)	28 (33)	30 (27)	7 (6)
non-CHD	71	38 (53)	27 (38)	4 (6)	1 (1)

Serum total lipids

A similar ratio between the groups was apparent for serum total lipids as well (Table 16). The means rose with age in the same proportions in both CHD and non-CHD patients, so that similar differences were seen at each age group.

Hyperlipoproteinemias

About a third of the CHD patients, but over a half of the non-CHD patients, had normal lipid values which excluded any type of hyperlipoproteinemia (Table 17). The differences between the groups were most obvious for type II B ($p < 0.001$) whereas for types II A and IV they were small (p NS).

Serum alpha lipoprotein

The levels of serum alpha-lipoprotein were lower in all age groups of CHD patients compared to non-CHD patients, as also were the total group means (Table 16).

Table 16 Mean serum lipids in CHD and non-CHD patients. The differences between the groups were highly significant ($p < 0.001$)

Group	Cholesterol			Triglycerides			Total lipids			Alpha-lipoprotein		
	N	mmol/l	S.D.	N	mmol/l	S.D.	N	g/l	S.D.	N	g/l	S.D.
CHD	102	7.6	1.6	108	2.15	1.37	112	8.5	2.1	112	1.50	0.42
non-CHD	85	7.0	1.7	81	1.4	1.01	71	7.4	1.5	71	1.93	0.53

THE RELATIONSHIP OF THE EAR LOBE CREASE TO THE EXTENT OF CORONARY ATHEROSCLEROSIS

Single vessel disease

EC was seen in 26 of the 50 patients (52 %) with single vessel disease (Table 15) The prevalence was lowest at the ages of 30 to 39 in which group EC was found in only 4 of 14 patients (29 %) but the frequency tended to increase with age (Fig 14) At the ages of 40 to 49 EC was found in 13 of 22 patients (59 %) and in the 50 to 59 age group in 7 of 11 (64 %) Two of the 3 patients over 60 years had EC

Double vessel disease

The prevalence of EC also tended to increase with age in patients with double vessel disease (Fig 14) The prevalence was higher

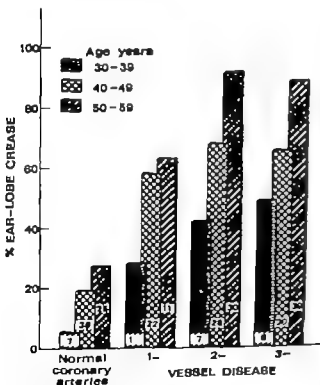


Fig 14 Prevalence of the ear lobe crease in patients studied by coronary angiography by age and extent of coronary atherosclerosis. The numbers in the columns indicate the numbers of all individuals in each group.

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Triple vessel disease

In triple vessel disease the prevalence of EC was fairly similar to that in double vessel disease. In the whole group 66 of 84 patients (79 %) had EC. At the ages of 30 to 39 it was seen in 3 of 6 patients (50 %) at 40 to 49 years in 20 of 30 patients (67 %) and at 50 to 59 years in 35 of 39 patients (90 %) The group also included 9 patients over 60 years, of whom 8 (89 %) had EC (Table 15 Fig 14). The prevalence of EC did not differ statistically significantly from that in single vessel disease.

PREVALENCE OF CORONARY RISK FACTORS IN CHD AND NON CHD PATIENTS

The following risk factors are described for the CHD and non-CHD groups, men and women combined. In addition, the comparisons have been done between the men of both groups too but this is mentioned only if the two sets of results differed from each other

Blood pressure

History of arterial hypertension

Hypertension had been diagnosed before this study in 31 CHD patients (31 %) and in 15 patients of the non-CHD group (17 %) The difference was significant ($p < 0.01$)

Systolic blood pressure

In the CHD group the mean systolic blood pressure was higher in all age groups than in the non-CHD group. For the whole groups the means were 137 ± 17 mmHg and 133 ± 18 mmHg, respectively ($p < 0.01$).

Diastolic blood pressure

The relation between the groups was similar for the diastolic blood pressure. The means were higher in the CHD than the non-CHD group. In the former group the mean was 88 ± 10 mmHg and in the latter 84 ± 9 mmHg. The difference was statistically significant ($p < 0.001$).

Serum lipids

Serum cholesterol

The mean serum cholesterol concentrations in the CHD and non-CHD groups are shown in Table 16. In different age groups the mean values were also significantly higher in the CHD group compared to the non-CHD group except between those aged 50 to 59 years.

Serum triglycerides

The mean serum triglyceride concentrations were elevated in all age groups of the CHD patients, but normal in all age groups of the non-CHD patients. The total means of the groups are presented in Table 16.

Table 17 Distribution of CHD and non-CHD patients according to type of hyperlipoproteinemia. Figures in parentheses indicate percentages

Group	No of pts.	Type of hyperlipoproteinemia			
		0	II A	II B	IV
CHD	112	38 (34)	28 (25)	30 (27)	7 (6)
non-CHD	71	38 (53.5)	37 (52)	4 (6)	1 (1.4)

Serum total lipids

A similar ratio between the groups was apparent for serum total lipids as well (Table 16). The means rose with age in the same proportions in both CHD and non-CHD patients, so that similar differences were seen at each age group.

Hyperlipoproteinemias

About a third of the CHD patients, but over a half of the non-CHD patients, had normal lipid values which excluded any type of hyperlipoproteinemia (Table 17). The differences between the groups were most obvious for type II B ($p < 0.001$), whereas for types II A and IV they were small (p NS).

Serum alpha lipoprotein

The levels of serum alpha-lipoprotein were lower in all age groups of CHD patients compared to non-CHD patients, as also were the total group means (Table 16).

Table 16 Mean serum lipids in CHD and non-CHD patients. The differences between the groups were highly significant ($p < 0.001$)

Group	Cholesterol			Triglycerides			Total lipids			Alpha-lipoprotein		
	N	mmol/l	S.D.	N	mmol/l	S.D.	N	g/l	S.D.	N	g/l	S.D.
CHD	183	7.8	1.8	189	2.15	1.37	112	8.8	2.1	112	1.60	0.43
non-CHD	85	7.0	1.7	81	1.24	1.01	71	7.4	1.5	71	1.83	0.53

Smoking

In the CHD group there were 83 current smokers (41.5 %) 104 ex smokers (52 %) and 13 non smokers (6.5 %). This distribution differed considerably from that in the non-CHD group ($p < 0.001$) in which non-smokers formed the largest classification with 42 patients (49 %) whereas smokers and ex smokers were distributed almost equally the former amounting to 20 (23 %) and the latter to 24 (28 %). The differences were smaller however between the groups of non-CHD men and CHD men. Among the non-CHD men there were 11 current smokers (31 %) 17 ex smokers (48 %) and 7 non-smokers (20 %).

Diabetes mellitus

Diabetes, or a pathological glucose tolerance, was verified more frequently in the CHD than in the non-CHD group. It was seen in 43 of the former group (21.5 %) and in only 8 (9 %) of the latter ($p < 0.05$).

Obesity

The mean relative body weight was 0.255 ± 0.031 in the CHD group and 0.245 ± 0.030 in the non CHD group. The means also differed in a similar way ($p < 0.001$) in all age groups except for that over 60 years.

Blood group

The blood group was determined in 172 CHD patients and 65 non-CHD patients. There were 60 CHD patients (46.5 %) of blood group A, 30 (17 %) of B, 14 (8 %) of AB and 48 (28 %) of O. The percentages for the non-CHD patients were very close (p NS) to those (41.5 %, 23 %, 7.7 %, and 27.7 %, respectively).

Family history of premature cardiovascular disease and diabetes. Birthplace

Premature CHD in the family was recorded for 152 patients of the CHD group (hypertension for 73 to them (36.5 %) diabetes for 47 (23.5 %).

In the non-CHD group by comparison prevalences of CHD and hypertension family differed for the whole group and the men only. In the whole group 52 p (60.5 %) had CHD in the family the prevalence being somewhat lower for men of 35 (46 %). Similarly the prevalence of hypertension in the family was higher in the whole group than for men only. Six patients in the whole group (53.5 %) 13 men (37 %) had answered in the affirmative on this point. Diabetes existed in the families of 25 non-CHD patients (29 %).

With regard to CHD the difference between the groups was statistically significant ($p < 0.01$) to hypertension almost significant ($p < 0.05$) and to diabetes non-significant.

In the CHD group 91 patients (46 %) were born in eastern Finland, 74 (37 %) in western Finland and 33 (17 %) in Karelia. The geographical distribution was similar (p NS) in the non CHD group in which 37 patients (43 %) were born in eastern Finland, 38 (46 %) in western Finland and 16 (19 %) in Karelia. The distributions by birthplace of the parents corresponded well with the

THE DIAGONAL EAR LOBE CREASE AND CORONARY RISK FACTORS

The comparison covered the CHD and non-CHD patients as separate groups. Both groups were likewise further divided into patients with the bilateral or unilateral EC (EC patients) and those without EC (non-EC patients).

Coronary risk factors in CHD patients with and without the car-lebo crease

The CHD group included 144 EC patients (72 %) and 56 NEC patients (28 %), as described more precisely earlier in this paper. The statistical analysis was done between the total groups and also in different age groups.

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Blood pressure

History of arterial hypertension

Hypertension had been previously diagnosed in 24 % of the EC and in 23 % of the NEC patients. The difference was not significant.

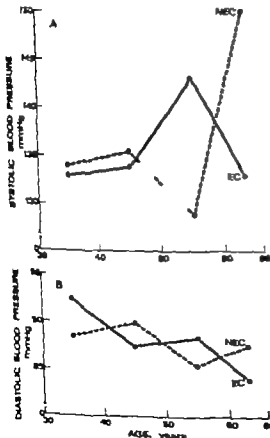


Fig 13 Mean systolic (A) and diastolic (B) blood pressure of EC and NEC patients in the CHD group

Systolic blood pressure

The mean systolic blood pressure was 138 ± 18 mmHg in the EC and 135 ± 15 mmHg in the NEC patients (p NS). The largest differences were between the 50 to 60 year age groups and those over 60 years, though in opposite directions (Fig 15).

Diastolic blood pressure

The mean diastolic blood pressure was almost equal in the EC and NEC groups, being 83 ± 11 mmHg in the former and 89 ± 9 mmHg in the latter. The differences by age groups were also small and not significant (Fig 15).

Serum lipids

Serum cholesterol

The mean serum cholesterol values of the entire groups were similar at 7.8 ± 1.6 mmol/l in the EC and 7.7 ± 1.5 mmol/l in the NEC patients. By age groups the means varied to both sides of a hypothetical horizontal plane (Fig 16), but again without statistical significance.

Serum triglycerides

Differences in mean serum triglycerides were statistically non-significant. In the EC patients the mean was 2.2 ± 1.6 mmol/l and in the NEC patients 2.1 ± 2.1 mmol/l. The values by age groups are presented in Fig. 17.

Serum total lipids

The mean serum total lipid value was the same, 8.5 g/l, in the EC (S.D. ± 2.2) and in the NEC patients (S.D. ± 1.5), but in the two

Smoking

In the CHD group there were 83 current smokers (41.5 %) 104 ex smokers (52 %) and 13 non-smokers (6.5 %) This distribution differed considerably from that in the non-CHD group ($p < 0.001$) in which non smokers formed the largest classification with 42 patients (49 %) whereas smokers and ex smokers were distributed almost equally the former amounting to 20 (23 %) and the latter to 24 (28 %) The differences were smaller however between the groups of non-CHD men and CHD men. Among the non-CHD men there were 11 current smokers (31 %) 17 ex smokers (49 %) and 7 non smokers (20 %)

Diabetes mellitus

Diabetes, or a pathological glucose tolerance, was verified more frequently in the CHD than in the non-CHD group It was seen in 4% of the former group (21.5 %) and in only 8 (9 %) of the latter ($p < 0.05$)

Obesity

The mean relative body weight was 0.235 ± 0.031 in the CHD group and 0.245 ± 0.030 in the non-CHD group The means also differed in a similar way ($p < 0.001$) in all age groups except for that over 60 years.

Blood group

The blood group was determined in 172 CHD patients and 65 non-CHD patients. There were 80 CHD patients (46.5 %) of blood group A, 30 (17 %) of B 14 (8 %) of AB and 48 (28 %) of O The percentages for the non-CHD patients were very close (p NS) to these (41.5 %, 23 %, 7.7 %, and 27.7 %, respectively)

Family history of premature cardiovascular disease and diabetes Birthplace

Premature CHD in the family was recorded for 152 patients of the CHD group (76 %) hypertension for 73 to them (36.5 %) and diabetes for 47 (23.5 %)

In the non-CHD group, by comparison, the prevalences of CHD and hypertension in the family differed for the whole group and for the men only In the whole group 52 patients (60.5 %) had CHD in the family the prevalence being somewhat lower for men — 16 of 35 (46 %) Similarly the prevalence of hypertension in the family was higher for the whole group than for men only Forty six patients in the whole group (53.5 %) and 13 men (37 %) had answered in the affirmative on this point. Diabetes existed in the families of 26 non-CHD patients (29 %)

With regard to CHD the difference between the groups was statistically significant ($p < 0.01$) to hypertension almost significant ($p < 0.05$) and to diabetes non significant.

In the CHD group 91 patients (46 %) were born in eastern Finland, 74 (37 %) in western Finland and 33 (17 %) in Karelia. The proportional distribution was similar (p NS) for the non-CHD group in which 37 patients (43 %) were born in eastern Finland, 33 (38 %) in western Finland and 16 (19 %) in Karelia. The distributions by birthplace of the parents corresponded well with these.

THE DIAGONAL EAR LOBE CREASE AND CORONARY RISK FACTORS

The comparison covered the CHD and non-CHD patients as separate groups. Both groups were likewise further divided into patients with the bilateral or unilateral EC (EC patients) and those without EC (NEC patients)

Coronary risk factors in CHD patients with and without the ear lobe crease

The CHD group included 144 EC patients (72 %) and 56 NEC patients (28 %), as described more precisely earlier in this paper. The statistical analysis was done between the total groups and also in different age groups.

Blood pressure

History of arterial hypertension

Hypertension had been previously diagnosed in 34 % of the EC and in 23 % of the NEC patients. The difference was not significant.

Systolic blood pressure

The mean systolic blood pressure was 138 ± 18 mmHg in the EC and 135 ± 13 mmHg in the NEC patients (p NS). The largest differences were between the 50 to 59 year age groups and those over 60 years, though in opposite directions (Fig. 15).

Diastolic blood pressure

The mean diastolic blood pressure was almost equal in the EC and NEC groups, being 88 ± 11 mmHg in the former and 89 ± 9 mmHg in the latter. The differences by age groups were also small and not significant (Fig. 15).

Serum lipids

Serum cholesterol

The mean serum cholesterol values of the entire groups were similar at 7.8 ± 1.6 mmol/l in the EC and 7.7 ± 1.5 mmol/l in the NEC patients. By age groups the means varied to both sides of a hypothetical horizontal plane (Fig. 16), but again without statistical significance.

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Differences in mean serum triglycerides were statistically non-significant. In the EC patients the mean was 2.2 ± 1.6 mmol/l and in the NEC patients 2.1 ± 2.1 mmol/l. The values by age groups are presented in Fig. 16.

Serum total lipids

The mean serum total lipid value was the same, 8.5 g/l, in the EC ($S.D. \pm 2.2$) and in the NEC patients ($S.D. \pm 1.8$), but in the two

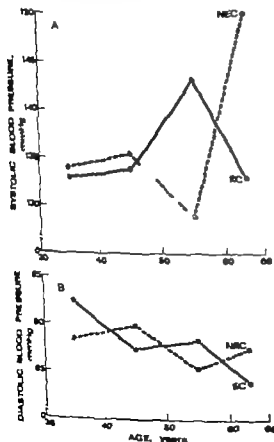


Fig. 15 Mean systolic (A) and diastolic (B) blood pressure of EC and NEC patients in the CHD group.

Smoking

In the CHD group there were 83 current smokers (41.5 %) 104 ex smokers (52 %) and 13 non smokers (6.5 %). This distribution differed considerably from that in the non-CHD group ($p < 0.001$) in which non smokers formed the largest classification with 42 patients (49 %) whereas smokers and ex smokers were distributed almost equally the former amounting to 20 (23 %) and the latter to 24 (28 %). The differences were smaller however between the groups of non-CHD men and CHD men. Among the non-CHD men there were 11 current smokers (31 %), 17 ex smokers (49 %) and 7 non smokers (20 %).

Diabetes mellitus

Diabetes or a pathological glucose tolerance, was verified more frequently in the CHD than in the non-CHD group. It was seen in 43 of the former group (21.5 %) and in only 8 (9 %) of the latter ($p < 0.05$).

Obesity

The mean relative body weight was 0.265 ± 0.031 in the CHD group and 0.245 ± 0.030 in the non-CHD group. The means also differed in a similar way ($p < 0.001$) in all age groups except for that over 60 years.

Blood group

The blood group was determined in 172 CHD patients and 85 non-CHD patients. There were 80 CHD patients (46.5 %) of blood group A, 30 (17 %) of B, 14 (8 %) of AB, and 48 (28 %) of O. The percentages for the non-CHD patients were very close (p NS) to these (41.5 %, 23 %, 7.7 %, and 27.7 %, respectively).

Family history of premature cardiovascular disease and diabetes Birthplace

Premature CHD in the family was recorded for 152 patients of the CHD group (76 %) hypertension for 73 to them (36.5 %) and diabetes for 47 (23.5 %).

In the non-CHD group by comparison, the prevalences of CHD and hypertension in the family differed for the whole group and for the men only. In the whole group 52 patients (60.5 %) had CHD in the family the prevalence being somewhat lower for men — 16 of 35 (46 %). Similarly the prevalence of hypertension in the family was higher for the whole group than for men only. Forty six patients in the whole group (53.5 %) and 13 men (37 %) had answered in the affirmative on this point. Diabetes existed in the families of 25 non-CHD patients (29 %).

With regard to CHD the difference between the groups was statistically significant ($p < 0.01$) to hypertension almost significant ($p < 0.05$) and to diabetes non significant.

In the CHD group 91 patients (46 %) were born in eastern Finland, 74 (37 %) in western Finland and 33 (17 %) in Karelia. The proportional distribution was similar (p NS) for the non-CHD group, in which 37 patients (43 %) were born in eastern Finland, 33 (38 %) in western Finland and 16 (19 %) in Karelia. The distributions by birthplace of the parents corresponded well with these.

THE DIAGONAL EAR LOBE CREASE AND CORONARY RISK FACTORS

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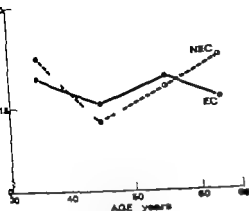


Fig. 19 Mean serum alpha-lipoprotein of EC and NEC patients in the CHD group.

9). The total means for the two groups were the same at 1.4 ± 0.4 g/l, so that a statistically significant difference was absent in this case, too.

Smoking

Among the EC patients there were 42 / current smokers, 52 ex smokers and 7 non-smokers, and these proportions were fairly similar for the NEC patients (41 /, 54 %, and 5 %). The differences were not statistically significant.

Diabetes mellitus

Diabetes, or at least a pathological glucose tolerance, was almost as frequent in the EC (21 %) as in the NEC patients (23 %), and again there was no statistically significant difference.

Obesity

The mean relative body weight was 0.236 ± 0.032 in the EC and 0.251 ± 0.029 in the NEC patients. This difference was not significant and neither were those for all age groups (Fig. 20).

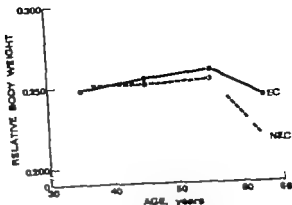


Fig. 20 Mean relative body weight of EC and NEC patients in the CHD group.

Blood group

The proportional differences between the EC and NEC groups were greatest for the blood groups A and O. There were more EC (49 %) than NEC patients (40 %) of blood group A, whereas the prevalence was reversed for group O which included 25 % of the EC and 38 % of the NEC patients. The frequencies of blood groups B and AB differed little between the EC (18 % and 9 %) and NEC patients (17 % and 6 %). All differences were statistically non-significant.

Family history of premature cardiovascular disease and diabetes. Birthplace

Differences in the family histories of the investigated diseases — CHD, hypertension, and diabetes — did not reach statistical significance in any respect. The relative proportions were, for CHD 76 % in the EC and 73 % in the NEC patients, for hypertension 33 % and 39 %, and for diabetes 23 % and 20 %, respectively.

Both the CHD patients and their parents in each group were distributed very similarly by birthplace. None of the regions showed statistically significant differences between the EC and NEC patients (Fig. 21).

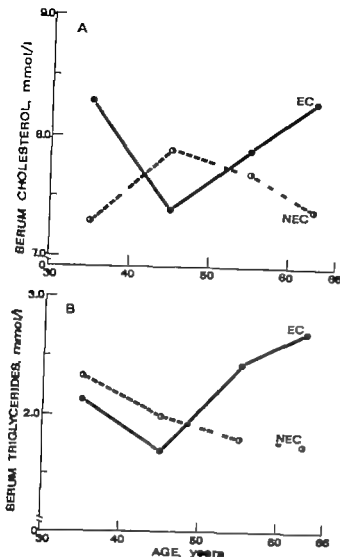


Fig. 16 Mean serum cholesterol (A) and triglycerides (B) of EC and NEC patients in the CHD group.

youngest age groups the values were higher in the EC than NEC patients, and this situation was reversed in the two older groups (Fig 17). However these differences were not statistically significant.

Hyperlipoproteinemias

The proportional distribution of different classes of hyperlipoproteinemia (II A, II B and IV) was fairly uniform among both the EC and NEC patients (Fig 18), and failed to demonstrate any statistically significant differences.

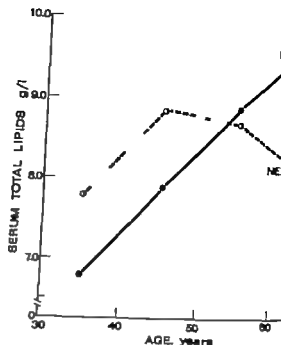


Fig 17 Mean serum total lipids of EC and patients in the CHD group.

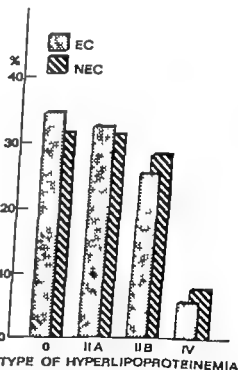


Fig 18 Distribution of EC and NEC patients in the CHD group according to type of hyperlipoproteinemia.

Serum alpha lipoprotein

The EC and NEC patients resembled each other in the serum alpha lipoprotein range which was narrow in all age groups (Fig

THE HISTOPATHOLOGIC POSTMORTEM STUDY OF THE EAR LOBE

The postmortem study was an attempt to determine whether any definite histopathologic changes in the lobule portion of the auricle could be found to explain the formation of EC.

The ear lobes of 10 patients who had died from acute myocardial infarction were studied. The findings were compared to those of 10 age-matched control patients who died from a cause other than cardiovascular disease and were free of significant coronary atherosclerosis at autopsy. All patients with myocardial infarction had EC, whereas all control subjects were without EC. The age of both groups ranged from 43 to 65 years, with a mean of 56 years. There was only one woman in each group and both were 65 years old.

The samples from the lobules of the auricles were fixed in formalin. The sections were stained with hematoxylin-eosin, Weigert's elastic tissue stain and Gomori's reticulum stain. The specimens were examined by two experienced pathologists, independently. The findings from the ear lobes included distended capillaries, some unspecific inflammatory changes around these and collagen degeneration being of the type caused by light. These features did not differ between the groups apart from the presence of more advanced acute stasis in patients with myocardial infarction. A single exception was a myocardial infarction patient aged 56 who also had significant sclerosis of the small arteries of the lobule of the auricle.

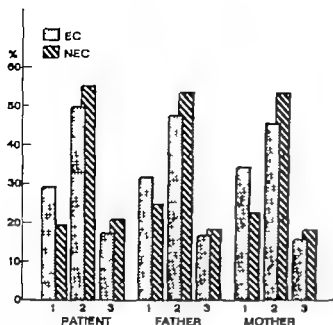


Fig 21 Distribution of EC and NEC patients and their parents in the CHD group according to birthplace (1. west Finland, 2. east Finland, 3. Karelia)

Other characteristics of CHD patients with and without the ear lobe crease

Previous and current history of CHD

Angina pectoris had been experienced for 58 ± 4.1 years by the EC patients and for 40 ± 3.0 years by the NEC patients. This difference was not statistically significant, and neither were those in all age groups. However symptomatic CHD had been present for longer in the EC than in the NEC patients.

There had been one or more myocardial infarction in 61 % of both EC and NEC patients, and no significant differences existed with respect to the numbers of previous infarctions, either

Coronary by pass operation had been performed in 35 % of the EC patients and in 30 % of the NEC patients (p NS)

Other cardiovascular disease

In the CHD group 33 patients (16.5 %) had some other cardiovascular disease, of which intermittent claudication was most general, involving 24 patients. Other diseases involved were stenosis of the neck arteries (5), atrial septal defect (2) and aortic stenosis (3). A previous history of a disease of this kind was recorded in 29 % of the EC and in 7 % of the NEC patients, again a difference which did not reach statistical significance.

Drug treatment

The use of beta blockers and serum lipid lowering agents (clofibrate being the most important) was relatively less common among the EC (64 % and 13 %) than among the NEC patients (75 % and 21 %). These small differences were not, however statistically significant.

Coronary risk factors in non-CHD patients with and without the ear lobe crease

The non-CHD group was small compared with the others, being made up of 86 patients, — 18 (21 %) with EC and 68 (79 %) without EC. The statistical evaluation of the relationship between EC and coronary risk factors was only made between total EC and NEC patients, disregarding age and sex.

The differences in the distribution of risk factors between the EC and NEC patients were not statistically significant, with the exception of previous myocardial infarction. This had been diagnosed prior to the study in 6 of 86 non-CHD patients (7 %) of whom only one was without EC. This interesting difference between the EC and NEC patients was statistically significant ($p < 0.01$).

THE HISTOPATHOLOGIC POSTMORTEM STUDY OF THE EAR LOBE

The postmortem study was an attempt to determine whether any definite histopathologic changes in the lobule portion of the auricle could be found to explain the formation of EC.

The ear lobes of 10 patients who had died from acute myocardial infarction were studied. The findings were compared to those of 10 age-matched control patients who died from a cause other than cardiovascular disease and were free of significant coronary atherosclerosis at autopsy. All patients with myocardial infarction had EC, whereas all control subjects were without EC. The age of both groups ranged from 43 to 63 years, with a mean of 56 years. There was only one woman in each group and both were 63 years old.

The samples from the lobules of the auricles were fixed in formalin. The sections were stained with hematoxylin-eosin, Weigert's elastic tissue stain and Gomori's reticulum stain. The specimens were examined by two experienced pathologists, independently. The findings from the ear lobes included distended capillaries, some unspecific inflammatory changes around these and collagen degeneration being of the type caused by light. These features did not differ between the groups apart from the presence of more advanced acute stasis in patients with myocardial infarction. A single exception was a myocardial infarction patient aged 56 who also had significant sclerosis of the small arteries of the lobule of the auricle.

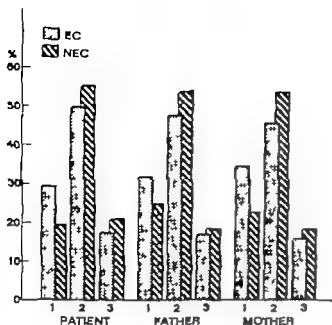


Fig 21 Distribution of EC and NEC patients and their parents in the CHD group according to birthplace (1 west Finland, 2 east Finland, 3 Karelia)

Other characteristics of CHD patients with and without the ear lobe crease

Previous and current history of CHD

Angina pectoris had been experienced for 58 ± 4.1 years by the EC patients and for 40 ± 3.0 years by the NEC patients. This difference was not statistically significant, and neither were those in all age groups. However symptomatic CHD had been present for longer in the EC than in the NEC patients.

There had been one or more myocardial infarction in 61 % of both EC and NEC patients, and no significant differences existed with respect to the numbers of previous infarctions, either.

Coronary bypass operation had been performed in 35 % of the EC patients and in 30 % of the NEC patients (p NS).

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Dear et al. 1971 Eliot et al. 1974). With these limitations in mind the control subject material of the present study should meet the requirements, being an age- and sex matched sample of the normal population, free of clinical coronary heart disease. Among the patients evaluated angiographically the group with normal coronary arteries was also small in the present study although larger than in any previous one.

In the present study the diagonal ear lobe crease was found to be present in a remarkably high proportion of patients with myocardial infarction, viz. 69 per cent of the total group and 68 per cent of those under 60 years of age, and there was a trend of correlation with the age of the patients. On the other hand, only 25 per cent of the age- and sex-matched control subjects had this sign. The prevalence of the ear lobe crease in this Finnish coronary heart disease material was thus high compared with previous similar studies (Lichstein et al. 1974, Christiansen et al. 1975), especially since those latter investigations included rather old patients, up to 89 years of age. This is noteworthy because it seems that the prevalence of the ear lobe crease increases as a function of age, which has led to the suggestion that it is associated merely with advanced age (Siehla and Hamby 1974). However in the present study the prevalence of the ear lobe crease was fairly high in all age groups of patients with acute myocardial infarction, even though it also tended to increase with age in these patients, too. The ear crease was already fairly common below 50 years of age, and the increase above 50 occurred smoothly without showing any sharp rise. Sex did not appear to cause differences in the prevalence of the ear crease of these patients at any age.

In the control subjects of the present study a similar increasing trend in the prevalence of the ear lobe crease in relation to age was observed in men, whereas in the women the

sign was rather infrequent and detectable earliest at the age of 57 years. Both asymptomatic and symptomatic coronary atherosclerosis is well known to occur more frequently in young and middle-aged men and to progress more rapidly than in women of the same age (Blumgart et al. 1940 White et al. 1950 Zolf et al. 1951 Allison et al. 1963, Eggen and Solberg 1968 Strong et al. 1968, Tejada et al. 1968 Welch et al. 1970 and 1975). However in the present study the ear lobe crease was significantly less frequent in the age-matched sample of the normal population than in patients with acute myocardial infarction, and the effect of age did not seem to obscure the true positive association between the ear lobe crease and coronary heart disease.

Furthermore, these observations are strongly supported by the present findings in the patients with coronary heart disease documented by coronary angiography where the prevalence of the ear-lobe crease under 60 years of age (71 %) was similar to that in the patients with acute myocardial infarction. Also, the prevalence of the ear crease in the patients with normal coronary arteries or only insignificant coronary sclerosis (19 %) resembled that of the control subjects.

In addition to this, the diagonal ear lobe crease was more frequent in patients with more extensive coronary atherosclerosis. Lichstein et al. (1976) observed a similar trend while examining the association of the ear crease with the postmortem findings in coronary arteries. In their study the extent of coronary atherosclerosis in 39 patients with the bilateral ear crease was significantly more severe compared with the arteries of 32 patients with no ear crease. In the present study the ear crease was present in 52 per cent of the patients with single vessel disease and in 79 per cent of those with double or triple vessel disease. Even in those with only double vessel disease coronary angiography usually revealed

DISCUSSION

Since the discovery of the diagonal ear lobe crease, attempts have been made to clarify its significance and relationship to coronary heart disease. In the course of these investigations the ageing process has been found to have a prominent role of its own in this context.

Frank (1973) suggested that the ear lobe crease might be associated with premature cardiovascular disease. Lichstein et al. (1974) regarded it as a coronary risk factor as did Christiansen et al. (1975) on the basis of their study which resembled the former one. The ear crease was found to be infrequent under 50 years of age and thereafter to increase markedly especially in the latter study. The relationship of the ear crease to coronary heart disease, as confirmed by coronary angiography has been investigated by Sternlieb et al. (1974). They considered the relationship to be positive, whereas Mehta and Hamby (1974) suggested, on the basis of a similar material, that the ear lobe crease was correlated only with advancing age. The sign has also been noticed in connection with diabetic retinopathy and this has been presumed to indicate a correlation with generalized angiopathy (Andresen et al. 1976). Sprague (1976) considered the ear lobe crease to be a useful indicator of an operative risk and also to be related to coronary heart disease. Findings have thus been indicative of a positive relationship although the real nature of the diagonal ear lobe crease remains to be clarified and even its basic significance

is still strongly suspected by some. The undoubted effect of age, in particular is the most important challenge to this possible relationship.

Forming a comparable patient and control material is obviously the main and most important difficulty in clinical research on coronary heart disease. The diagnosis of coronary heart disease, whether assessed by acute myocardial infarction or coronary angiography does not raise difficulties. The symptoms and signs of acute myocardial infarction are usually obvious and can be defined. Coronary angiography has also proved a reliable tool in the evaluation of coronary atherosclerosis, as shown by many comparative studies of postmortem findings (e.g. Kemp et al. 1967 Vlodaver et al. 1973, Grondin et al. 1974 Schwartz et al. 1975). The main difficulties arise in obtaining suitable control material. Subjects with no clinically demonstrable coronary heart disease are indeed easy to find, but some such individuals may have significant atherosclerotic changes in their coronary arteries (Enos et al. 1953, McNamara 1971). For ethical reasons coronary angiography cannot be done in a symptomless individual because of the risk involved. The proportion of subjects with angiographically normal coronary arteries is therefore bound to remain small. Some of these patients may, in addition, suffer from another heart disease or ischemic heart disease without coronary atherosclerosis (e.g. Campeau et al. 1968,

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In addition to this, the diagonal ear lobe crease was more frequent in patients with more extensive coronary atherosclerosis. Lichstein et al. (1978) observed a similar trend while examining the association of the ear crease with the postmortem findings in coronary arteries. In their study the extent of coronary atherosclerosis in 59 patients with the bilateral ear crease was significantly more severe compared with the arteries of 33 patients with no ear crease. In the present study the ear crease was present in 82 per cent of the patients with single vessel disease and in 79 per cent of those with double or triple vessel disease. Even in those with only double vessel disease coronary angiography usually revealed extensive coronary athero-

sclerosis, even though significant occlusive lesions were restricted to two main arteries.

Myocardial infarction had been previously diagnosed in 7 per cent of the patients with normal coronary arteries in the present study. In the literature 11 to 16 per cent of all myocardial infarctions have been reported to occur in patients who do not demonstrate angiographic evidence of significant coronary atherosclerosis (e.g. Campeau et al. 1968, Bruschke et al. 1971, Dear et al. 1971, Glancy et al. 1971, Kimbiris et al. 1972, Potts et al. 1972, Elliot et al. 1974, Thompson et al. 1977). The fact that 5 of the present 6 patients with past infarction and normal coronary arteries had the ear lobe crease deserves consideration. None of these patients had any other cardiovascular disease. Frank (1977) has also observed an association between the ear crease and ischemic heart disease without coronary atherosclerosis.

The presence of the ear lobe crease did not correlate with the duration of angina pectoris nor with the number of previous infarctions in the patients with acute myocardial infarction. Neither did the severity of the present myocardial infarction — either transmural or non transmural — influence the matter. However angina pectoris showed a trend of longer duration in patients with angiographically documented coronary atherosclerosis and the ear lobe crease than in those without it, while the number of previous infarctions was fairly similar in both groups.

Apart from evaluating the relationship between the diagonal ear lobe crease and coronary heart disease the present study attempted to evaluate the relation of coronary risk factors to the ear lobe crease. On the basis of many prospective epidemiological studies (Keys 1970, Gertler and White 1976) coronary risk factors may definitely be said to increase the average risk of early coronary heart disease in both sexes, even though no strictly causal relationship has been established. The present study also indicated a

greater prevalence of risk factors in patients with coronary heart disease than in controls, but none of the coronary risk factors was established as being related to the presence of the diagonal ear lobe crease when analyzed separately in patients with myocardial infarction, control subjects and patients studied by coronary angiography. With regard to hypertension, diabetes and cigarette smoking Lichstein et al. (1974) have come to a conclusion of the same kind. Quite recently Rhoads et al. (1977) suggested that the ear lobe crease correlated with blood pressure and obesity but not with coronary heart disease.

None of the major coronary risk factors in the present study was associated with the ear lobe crease, — neither history of arterial hypertension, blood pressure values, cigarette smoking nor serum lipids. Serum lipids, especially elevated serum cholesterol, have been implicated in promoting the progression of coronary atherosclerosis (Bemis et al. 1973, Ben xvi et al. 1974), though not all observations favor this suggestion (Kimbiris et al. 1974, Frick et al. 1975, McLaughlin et al. 1977). In the present study the levels of serum lipids did not correlate with ear lobe crease though the patients with acute myocardial infarction of all age groups with the ear crease showed a trend towards higher means for serum cholesterol than those without it. The level of serum alpha lipoprotein was higher in the non-CHD than the CHD group, but not so in the patients without ear crease compared to those with it. Diabetes, the level of physical activity at work and during leisure time, obesity, blood group and family history of coronary heart disease or diabetes were not associated with the ear crease either. A history of hypertension in the families of the patients with myocardial infarction but without ear crease was obtained significantly more frequently than in those with the ear crease. However in the other groups no such relationship was seen. Rissanen (1974) has demonstrated that although

coronary heart disease is, in terms of both morbidity and mortality more prevalent in eastern than western Finland, there is no significant difference in the extent of coronary atherosclerosis between groups from these regions. The birthplace of the patients and subjects and their parents in the present study seemed to have no effect on the prevalence of the ear lobe crease, either

The possibility that the additive effect of the main risk factors known to have predictive value in individual subjects (Kannel et al. 1976) could still be related to the presence of the ear crease seems improbable, as the mean values of each risk factor in different age groups of the present study were rather similar. This was further checked by calculating the risk score using the data of the Framingham study in the largest age groups of the present study. No difference was observed between those with and without the ear crease. A discriminant function analysis of the risk factors in relation to the presence of the ear lobe crease is in progress.

The possibility of the ear lobe crease being associated with the drug treatment of coronary heart disease was not supported by the present results. Beta-blockers, especially practolol, have been reported to cause side effects in the skin. These include a lupus erythematosus-like syndrome (Raferty and Denman 1973) and eczema, exfoliative dermatitis, lichen planus, and a psoriasis-like rash (Felix et al. 1974). The present study did not suggest the ear-lobe crease to be a possible side effect of beta-blockers or other drugs.

The present results allow some speculation about the formation of the ear lobe crease and its relation to the age of the individual and the extent of coronary heart disease. It seems probable that the ear-lobe crease often starts unilaterally and later develops bilaterally and earlier in men than in women. The unilateral ear crease is fairly frequent compared with the bilateral sign in men under 50 years of age and in women up to 60 years, after which the bilateral sign becomes

dominant. On the other hand, the prevalence of the ear crease seems related to the severity and extent of coronary atherosclerosis, which in turn also usually correlates with the age of the patients, though with frequent exceptions. This might favor the view that both the ear crease and coronary heart disease share biological age as their common dominator.

In the present study the youngest patient with coronary heart disease was 25 years old, and he had the ear crease. Sprague (1976) has found this sign in still younger patients, aged 3, 14, and 17 years. There is some evidence that pathological changes leading to coronary heart disease might be initiated very early in childhood. In studies on the coronary arteries of infants and children of parents with a high hereditary risk of coronary heart disease, intimal and musculo-elastic layer thickenings in coronary arteries have been reported (Neufeld et al. 1962, Vlodaver et al. 1969, Pesonen 1974). These presumably genetically transmitted changes may predispose to premature coronary atherosclerosis.

The development of coronary arteries precedes that of the ear lobule. Therefore, simultaneous embryological mis-development cannot be proposed as a basis for a later association of the ear-lobe crease with coronary heart disease. A common genetic factor responsible for both the ear crease and coronary atherosclerosis could be speculated upon, though. Certain anthropological facts deserve attention in this respect. Halonen (1938) has found Finns to differ considerably from Japanese in the form of branching of coronary arteries and in the vascularization areas of these in the myocardium. The present study indicates that the prevalence of the ear lobe crease also differs considerably between Finns and Japanese with coronary heart disease. In the 50 to 59 year age group it appeared in 71 per cent of Finnish men, but in only 23.1 per cent of Japanese men (Rhoads et al. 1977), the latter figure corresponding

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The development of coronary arteries precedes that of the ear lobule. Therefore, simultaneous embryological mis-development cannot be proposed as a basis for a later association of the ear-lobe crease with coronary heart disease. A common genetic factor responsible for both the ear crease and coronary atherosclerosis could be speculated upon, though. Certain anthropological facts deserve attention in this respect. Halonen (1938) has found Finns to differ considerably from Japanese in the form of branching of coronary arteries and in the vascularization areas of these in the myocardium. The present study indicates that the prevalence of the ear lobe crease also differs considerably between Finns and Japanese with coronary heart disease. In the 60 to 59 year age group it appeared in 71 per cent of Finnish men, but in only 28.1 per cent of Japanese men (Rhoads et al. 1977), the latter figure corresponding

closest to the prevalence of the ear lobe crease in the control subjects of the present material. The same genetic factor to determine branching and vascularization areas of coronary arteries might also determine the susceptibility to coronary heart disease and the ear lobe crease.

The lobule of the auricle is covered by skin. The subcutaneous tissue is abundant and encloses numerous fat cells. The rich capillary bed and the relative paucity of nerve endings are characteristic features. The histopathologic changes in the investigated specimens were slight. Distended capillaries were more frequently found in the patients with myocardial infarction, but this reflects only the existence of a more general stasis in these patients. The significant atherosclerosis found in one specimen cannot be cause of the ear-lobe crease because the others were without similar changes. Adams et al. (1974) have noticed a correlation between coronary atherosclerosis and changes in the Achilles' tendon, and considered the cause to be aging avascular connective tissue. Some other studies indicate that collagen tissue might play a part in the pathogenesis of atherosclerosis (Cardinale and Udenfriend 1974

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Conclusion

The prevalence of the diagonal ear lobe crease is considerably higher in patients with acute myocardial infarction or coronary heart disease documented angiographically compared to control subjects with no clinically detectable coronary heart disease or to those with normal coronary arteries. In addition, the ear lobe crease is more frequent in the more severe cases of coronary atherosclerosis. In all groups the prevalence of the ear crease tends to increase with age. Coronary risk factors seem not to be associated with this sign. In a few patients the ear lobe crease was present in ischemic heart disease with normal coronary arteries. More studies are needed to clarify the precise value of the ear lobe crease as a clinical sign associated with coronary heart disease. A prospective study would be particularly useful in this respect.

SUMMARY

The purpose of the present study was to investigate the relationship of the diagonal ear lobe crease to coronary heart disease and coronary risk factors and, in addition, to evaluate the development of the ear lobe crease.

The clinical material consisted of 219 patients with acute myocardial infarction fulfilling the WHO criteria, 290 control subjects, and 238 patients studied by coronary angiography. The latter group comprised 200 CHD and 38 non-CHD patients. In each group, except for the non-CHD patients, women were in a minority. The age of the investigated patients and subjects ranged from 26 to 66 years, the majority being under 60.

Below 60 years of age the prevalence of the ear lobe crease was 66 % in the patients with myocardial infarction and 25 % in the control subjects — the corresponding values being 71 % in the CHD and 19 % in the non-CHD patients. The prevalence increased with advancing age in all groups without affecting the positive association between the ear-lobe crease and coronary heart disease. The bilateral sign became more dominant than the unilateral one with age, too.

The prevalence of the ear lobe crease also increased with the extent of coronary atherosclerosis. The ear crease was seen in 52 % of the patients with single vessel disease and 79 % of the patients with double or triple vessel disease. In the non-CHD patients the sign was found in 21 % of the total group. Five of 6 non-CHD patients with previously diagnosed myocardial infarction and normal coronary arteries had the ear crease.

The ear-lobe crease was not correlated with the duration of symptomatic coronary heart disease, and neither were any of the investigated established coronary risk factors positively related to the sign. These included heredity, the major risk factors — hypertension, hyperlipidemia, and cigarette smoking — and certain minor factors: diabetes, level of physical activity, obesity etc. The presence of other cardiovascular disease did not influence this sign either.

The postmortem study of ear-lobe samples from 10 patients with ear crease and acute myocardial infarction and from 10 controls without ear crease or signs of any cardiovascular disease revealed non-specific histopathologic changes in both groups.

closest to the prevalence of the ear lobe crease in the control subjects of the present material. The same genetic factor to determine branching and vascularization areas of coronary arteries might also determine the susceptibility to coronary heart disease and the ear lobe crease.

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By Ilkka Torstila



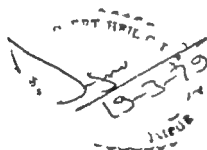
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Page 8	line 5: Hageman	read Hageman
11	14: precursos	precursor
26	7: glass tubes	plastic tubes
28	2: 1972	1971
28	30: in ice bath for 48 hours	in ice bath for 30 minutes and dialyzed against distilled water for 48 hours
29	22: guinea pig	guinea pig ileum

FROM THE WIIHURI RESEARCH INSTITUTE, HELSINKI,
AND FROM THE FIRST DEPARTMENT OF MEDICINE,
UNIVERSITY OF HELSINKI, FINLAND

The Plasma Kinin System in Acute Myocardial Infarction

BY
ILKKA TORSTILA

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INTRODUCTION

The pathophysiology of the acute manifestations of myocardial infarction is not yet fully understood in spite of extensive experimental and clinical research. Acute myocardial infarction is accompanied by disturbances in several neuro-humoral regulation mechanisms, including the sympatho-adrenergic, renin-angiotensin and histamine systems. In recent years, growing interest has been shown in the role of the plasma kallikrein-kinin system in acute coronary heart disease. Plasma kinins are among the most active biological substances which by inducing peripheral arterial vasodilatation cause a fall in blood pressure and locally cause pain. They also enhance capillary permeability and thus cause microcirculatory disturbances. Because of these well known facts plasma kinins have been thought to participate in the mechanisms of pain and circulatory collapse in acute myocardial infarction.

So far, however, the importance of the role of the plasma kinin system in this disease remains obscure. This is mainly due to difficulties in the methodology of kinin research as well as to difficulties in the interpretation of the observed changes in the kinin system in relation to the pathophysiological events.

The investigation presented here was designed to study the plasma kinin system in the acute phase of myocardial infarction with methodology partly developed in this laboratory.

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The investigation presented here was designed to study the plasma kinin system in the acute phase of myocardial infarction with methodology partly developed in this laboratory.

SURVEY OF THE LITERATURE

I REMARKS ON THE HISTORY AND CHEMISTRY OF THE KININ SYSTEM

In 1926-28 Frey and co-workers observed that human urine contains a substance that produces a prolonged fall in arterial blood pressure in the dog when injected intravenously. Kraut, Frey and Werle found a similar vasodepressor substance in human blood (1928) and in pancreas (1930) and assuming these substances to be identical, named the principle KALLIKREIN (Gr. $\kappa\alpha\lambda\lambda\iota\kappa\rho\epsilon\alpha\varsigma$ = pancreas).

Werle, Götze and Keppler discovered (1937) that kallikreins act indirectly by splitting off a pharmacologically active substance from a precursor present in blood. This substance was named KALLIDIN by Werle and Berek (1948). At the same time Rocha e Silva, Beraldo and Rosenfeld (1949) described the release of an active peptide from a plasma globulin fraction by trypsin and snake venoms. This peptide lowered blood pressure and also caused a slow contraction of the guinea pig ileum. Because of the slow response of the gut the substance was named BRADYKININ.

Soon thereafter it was shown that bradykinin and kallidin are derived from the same substrate (Werle and Berek 1950). Later kallidin was found to be lysylbradykinin (Pierce and Webster 1961). Bradykinin was isolated from ox blood by Elliot, Lewis and Horton (1960) and from human plasma by Hamberg (1962) and synthesized by Boissonnas, Guttman and Jaquenoud (1960).

Bradykinin, kallidin and another naturally occurring analogue, met-lys-bradykinin, are derived from the same precursor, KININOGEN by various KALLIKREINS (kininogenases). Met-lys-bradykinin has not yet been isolated in human plasma (Habermann 1970). The three peptides, brady-

Bradykinin is a nonapeptide with an amino acid sequence as shown in Fig 1. In plasma, kallidin is quickly converted to bradykinin by aminopeptidases (Webster and Pierce 1963).

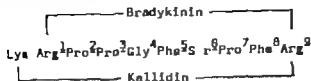


Fig 1 The amino acid sequence of human plasma kinins

The activation sequence of the human plasma kinin system (Fig 2) eventually leading to the activation of bradykinin involves a series of enzymes. The sequence begins with the activation of the Hageman factor (coagulation factor XII) and continues through stages which have so far been only partly explained until plasma prekallikrein is converted to active kallikrein.

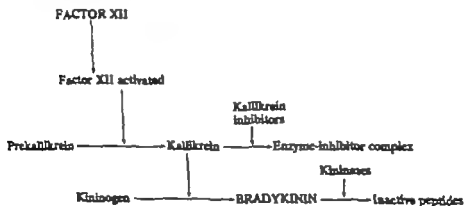


Fig 2 The activation sequence of the plasma kallikrein-kinin system

Margolis (1958) noted that glass contact results in release of kinins in human plasma and showed that this happens only in the presence of Hageman factor. Later studies have confirmed that Hageman factor is essential for kinin formation (Webster and Ratnoff 1961; Colman et al 1969, b). In addition to glass, there are several other substances and factors which have been pointed out as activators of Hageman factor in vitro: kaolin (Margolis 1963), collagen (Niewiarowski et al 1965), serum (Kossei 1966; Ogston et al 1969), chondroitin sulfate (Moskowitz

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that HK-kininogen are more susceptible to the action of plasma kallikrein which provides a potential control mechanism against direct bradykinin release (Colman 1974). Immunologically pure human kininogen was prepared by Hamberg and co-workers (Hamberg and Tallberg 1972; Hamberg et al 1975).

Active kinins are split very rapidly to inactive peptides by kinin destroying enzymes KININASES. There are two main locations for the inactivation of circulating kinins: plasma itself (Erdős 1967; Erdős and Sloane 1962) and the lungs (Ferreira and Vane 1967), which were shown to inactivate 80% of the injected bradykinin by one passage of blood. Because of the rapid inactivation, the half life of bradykinin is only 30-40 seconds, first shown by Samwell and Eskes (1962).

At present, two kininases are considered to account for the total kininase activity of plasma: kininase I and kininase II. Kininase I (arginine carboxypeptidase) (Erdős 1967) is primarily responsible for the hydrolysis of plasma kinins by removal of the C terminal arginine. Kininase II (peptidyl dipeptidase) (Yang and Erdős 1967) hydrolyzes the pro⁷ phe⁸ bond of bradykinin. From recent studies it is evident that kininase I functions mainly in plasma, while kininase II is the final terminator of kinin function in tissues, especially the lungs (Erdős 1976). It has been shown that kininase II is identical with angiotensin I converting enzyme which is known to function mainly in the lungs (Yang et al 1971).

II. RELATIONSHIP OF THE PLASMA KININ SYSTEM WITH OTHER PROTEOLYTIC ENZYME SYSTEMS

The activation of Hageman factor initiates not only the plasma kinin system but at least three other proteolytic enzyme systems in blood: i.e. the complement, coagulation and fibrinolytic systems (see Ratnoff 1974; Colman 1974). In addition to their activation by Hageman factor, all these systems have in common that a multitude of steps leads to the final active products, intrinsic amplification, several feedback mechanisms and finally common enzyme inhibition between complement, kinin and fibrinolytic systems by C1 esterase inhibitor (Ratnoff 1974) and coagulation- kinin and fibrinolytic systems by alpha 2 macroglobulin (Barrett and Starkey 1973).

It is noteworthy that activation of the plasma kinin system is further accelerated by plasmin, which degrades activated Hageman factor to the so called prekallikrein activators (PKA) (Kaplan and Austen 1971).

et al 1970} platelets (Walsh 1972) and urate crystals (Kellermeyer and Breckenridge 1965) among others

In general activators carry a negative charge (zeta potentials) and lose their activity after their negative potentials are neutralized (Haanen et al 1961 Eisen 1964 1969) Hageman factor activation has been demonstrated also by antibody antigen aggregates (Movat and Dilorenzo 1968) These as well as collagen may be important in the activation process. In certain disease states in vivo

Kallikreins are ubiquitous in the body and can be divided into tissue and plasma kallikreins In the submaxillary gland (Werle and von Roden 1936) and in the kidney (Frey 1926) the kallikreins are stored in an active form It has been shown that urinary kallikrein is identical with kidney kallikrein (Rustad 1970) Other tissue kallikreins are inert precursors molecules for which the activation mechanism has not yet been identified

Compared with tissue kallikreins (mol wt 24 000 - 36 000) human plasma kallikrein is of higher molecular weight (approx 100 000) and differs from them both physicochemically and immunologically It is synthesized as an inactive precursor PREKALLIKREIN by the liver (Werle et al 1955 Eisen and Vogt 1970) Plasma kallikrein liberates bradykinin and tissue kallikreins kallidin when acting on kininogen precursors (Webster and Pierce 1963 Habermann and Blennemann 1964)

Kallikreins were thought to be highly specific enzymes with only one natural substrate kininogen (Schachter 1969) but it now appears that kallikreins not only act as kininogenases but also play a role in other proteolytic mechanisms in plasma (see p 9)

Human plasma contains several KALLIKREIN INHIBITORS Ever since the discovery of kallikrein its action has been known to be partly inhibited (Kraut et al 1933) by an inhibitor in plasma which has later been shown to be identical with C I esterase inhibitor Other inhibitors of plasma kallikrein are alpha 2-macroglobulin alpha 1 antitrypsin and in the presence of heparin antithrombin III (Kagen et al 1963 Harpel 1970 McConnel 1972 Colman 1974 Harpel 1976)

It has been suggested that human plasma contains at least two classes of kininogens (see Habermann 1970) which are produced by the liver The first class includes the so called low molecular weight (LMW) kininogens I and II with a molecular weight of approximately 50 000 (Pierce and Webster 1966) In these two low molecular weight kininogens the bradykinin sequence of amino acids is located in different parts of the molecule The so called high molecular weight (HMW) kininogens (mol wt. approx. 200 000) are considered to be polymers of either of the two LMW kininogens (Colman et al 1971) They account for about 20% of the total

Despite the above mentioned observations no definite physiological roles may as yet be ascribed to the plasma kinin system

2 OVERALL CARDIOVASCULAR EFFECTS OF THE KININS

It has been claimed that the kinins possibly exert a direct positive inotropic action on the myocardium in vivo (Montague et al 1963 Reichgott and Melmon 1970) although this has recently been questioned (Hoshimoto et al 1977) It is difficult to distinguish the reflex sympatho-adrenergic activity as a consequence of hypotension from direct effects of the peptides

The effect of bradykinin on vascular tone in vivo depends on the nature of the vessel being investigated on the species on the dose of the peptide and especially on interaction with other endogenous vasoactive substances

Large doses of injected bradykinin release catecholamines through stimulation of adrenal medulla (Feldberg and Lewis 1964) but smaller doses act indirectly through baroreceptor reflexes (Mason and Melmon 1965; Staszewska Barczack and Vane 1967) Conversely epinephrine and norepinephrine seem to decrease plasma kininogen (Erdős 1961 Castania and Rotschild 1974) Nevertheless after epinephrine injection plasma kinin level does not increase but there is an increase in kininase activity (see Sicuteri 1970) The tendency of kinins to release catecholamines has led to the suggestion (Collier 1966) that the release is a protection mechanism of the body against systemic effects of the liberated kinins from the site of injury

It has also been shown that intravenous administration of bradykinin to man results in serotonin (5-hydroxytryptamine 5-HT) liberation (Peltoja 1972) and there is evidence that kinins can cause release of histamine (Melmon and Cline 1967) Conversely intravenous injection of histamine or histamine liberator 48/80 in man causes a decrease in plasma kininogen (see Sicuteri 1970 b)

Intravenous injection of bradykinin to mammals including man usually results in systemic arterial dilatation which has been thought to be a direct action of bradykinin (see Haddy et al 1970) In almost all species studied bradykinin dilates the majority of arterioles including the coronary arteries (see Brecher and Brodmann 1970)

The effects of bradykinin on postcapillary vasculature and pulmonary vasculature are varied Recently it has been suggested that

In the conversion of inactive Hageman factor to active form plasma kallikrein itself is even more potent than plasmin through a positive feedback mechanism which also confirms the intimate relationship between the plasma kinin system and coagulation and fibrinolytic systems (Kaplan et al 1976) Thus the role of kallikreins is not limited to their action as kininogenases

III PLASMA KININ SYSTEM IN PHYSIOLOGY AND PATHOLOGY

1 POSSIBLE PHYSIOLOGICAL ROLES

Because of their widespread distribution and their remarkable potency the kinins have been thought to play a significant role in normal physiology in mammals including man. Speculations on their possible function as chemical regulators of the blood flow of various organs are numerous. It has been suggested that the kinins are regulators of functional hyperemia in salivary, pancreatic, tongue and sweat glands (Hilton and Lewis 1956, Fox and Hilton 1958, Lewis 1967). Halmon et al (1968) supported by experimental work by Campbell et al (1968) and later by Assali et al (1971) have suggested that the plasma kinin system is responsible for the rapid circulatory changes at the time of birth resulting in conversion of the fetal circulation to the adult circulatory pattern.

It has also been suggested (Furuyama 1966) that the kinins act to regulate renal function. In normal human subjects infusion of bradykinin enhances renal blood flow, decreases glomerular filtration rate and increases urinary excretion of sodium (Webster and Gilmore 1964, Masjletti et al 1975). It is possible that the renal effects of the kinins are mediated through the action of prostaglandins (McGiff et al 1972, McGiff and Masjletti 1976).

Furthermore, activation of the plasma kinin system during exercise has been mentioned by Hilton and Lewis (1955), Fox and Hilton (1958) and Uchida and Ueda (1969). Malofiejew et al (cited by Lantsberg et al 1973) found a smaller increase in blood kallikrein activity during exercise in physically trained persons than in non trained ones. Lantsberg et al (1973) confirmed this finding and concluded that the blood kinin system evidently represents one of the humoral mechanisms governing the adaptation of the cardiovascular system to various conditions. Malofiejew (1973) has also suggested that the plasma kinin system is activated during the process of labor.

acute pancreatitis (Blomquist and Hamberg 1969; Sipilä 1977) postgastrectomy dumping syndrome (Wong et al 1974) allergic disorders (see Colman et al 1971) transfusion reactions (see Colman et al 1971) joint inflammation (see Webster and Haling 1970), and headache in migraine and subarachnoid hemorrhage (see Sicuteri 1970). In chronic alcoholic liver disease the synthesis of prekallikrein by the liver is reduced (Wong et al 1972). A 30-60% decrease in the normal kininogen level was shown to occur in plasma samples after thrombolytic treatment of deep venous thrombosis by streptokinase (Hamberg 1969).

It has been suggested that there is a connection between the vasodilator effects of the kinins and their effect on renal handling of sodium and water and the regulation of both normal and pathologic blood pressure. Streeten et al (1972) described an orthostatic syndrome probably caused by impaired destruction of circulating bradykinin. In patients with essential hypertension decreased excretion of urinary kallikrein has been observed (Elliot and Muzum 1934; Margolius et al 1971).

The participation of the plasma kinin system in acute myocardial infarction has been under investigation by several groups (see p. 15).

There are at least four hereditary disorders in which a lack of a component of the plasma kallikrein-kinin system has been observed. Lack of C1 esterase inhibitor which is also the principal kallikrein inhibitor in plasma has been detected in hereditary angioneurotic edema (Landerman et al 1962; Donaldson and Evans 1963). The absence of Hageman factor first described by Ratnoff and Colopy (1955) is an asymptomatic trait but may lead to disturbances in the activation of several proteolytic enzyme systems in plasma (Ratnoff 1974). Another clotting deficiency the Fletcher trait has been identified as being due to the absence of plasma prekallikrein (Muepper 1973; Weiss et al 1974). Recently a hereditary disorder with a blood clotting deficiency in vitro has been recognized by three groups and identified by spongy as Fitzgerald, Williams and Flautejac traits. The missing factor is believed to be a high molecular weight kininogen. These findings suggest that obviously also kininogen molecule plays a role in blood clotting (Weidmann and Abraham 1974; Colman et al 1975; Muepper et al 1975).

the different action of bradykinin on arterioles and venules is mediated through prostaglandin release (Terragno et al 1975) Accordingly bradykinin releases the vasodilator prostaglandin E from the arteries but liberates the vasoconstrictor prostaglandin F from the veins. The fact that indomethacin a known prostaglandin synthesis inhibitor blocks the vasodilator effect of bradykinin in the isolated perfused rabbit heart (Needleman et al 1975) further confirms this hypothesis.

The effect of bradykinin on blood pressure is biphasic. After the initial fall in pressure a secondary pressor phase often overshooting the control values is seen (see Haddy et al 1970). It has been shown that the pressor response is mediated via both central and peripheral sympathetic reflexes (see Haddy et al 1970). After the finding of the identity of angiotensin I converting enzyme and kininase II (Yang et al 1971) there has been increasing interest in their possible regulatory function on the plasma concentration of angiotensin II and bradykinin and regulation of normal blood pressure (see Mersey et al 1977).

The kinins increase capillary permeability to fluid and proteins. The action of increasing vascular permeability after intradermal injection of bradykinin was first shown by Holdstock et al (1957) in guinea pigs and later observed in man (Herxheimer and Schachter 1959). It has been suggested that the permeability increase is a purely mechanical phenomenon related to passive distension of the venules (Rowley 1964). However more recently it has been shown by Ferreira (1974) that prostaglandin E enhances the effect of bradykinin on capillary permeability and thus bradykinin might be a mediator of permeability increase through the release of prostaglandins.

3 KININS IN PATHOPHYSIOLOGY

The first clinical disorder in which kinins were suspected to play a significant role was the carcinoid syndrome (Oates et al 1964). It is possible that kinin action is responsible for the carcinoid flush in this disease. In different shock states including hemorrhagic anaphylactic, tourniquet and endotoxin shock the kinin system has been thought to be activated (see Vogel and Zickgraf Rüdel 1970). Most evidence of the activation of the kinins in shock has been found in endotoxin shock.

There is evidence of the activation of the plasma kinin system in

5 KININS AND PAIN

Bradykinin is among the most potent pain producing substances known causing pain when applied to the blister base in man (Armstrong 1957; Keele and Armstrong 1964) and pain and vasodilatation when injected into the brachial artery or into the carotid of man (Burch and dePasquale 1962 Sicuteri 1970) Intracoronary (Uchida et al 1971) and epicardial (Uchida and Muroo 1974 Staszewska Barczack et al 1976) administration of bradykinin in dogs produces pseudoeffective nociceptive responses. If before and during the injection or application of bradykinin a local circulatory arrest is produced the pain will be even more intense and prolonged (Sicuteri et al 1967).

It has been shown that bradykinin is released from the heart within minutes from the onset of hypoxia or ischemia (Furukawa et al 1969 Kura et al 1973). Thus it is plausible that bradykinin possibly along with other substances released locally at the site of injury is responsible for the pain as suggested originally by Lewis et al (1929-31) and later by Guzman et al (1964). The place of kinins in the pathogenesis of sustained painful states has also been questioned since cutaneous pain responses to kinins in man show marked tachyphylaxis (Korton 1963). However if the kinins are responsible for the pain in ischemia there are a number of factors that may potentiate their action in stimulating the sensory nerve endings. In circulatory arrest the distribution and removal of the peptide is limited. With gradual lowering of local pH the action of kininases on bradykinin is greatly decreased (Edery and Lewis 1962).

It has been suggested that the release and accumulation of serotonin (5-HT) may sensitize pain receptors to bradykinin (Sicuteri 1967). Furthermore it has been suggested that prostaglandins in conjunction with bradykinin may be responsible for the potentiation of pain sensitization in ischemia (Staszewska Barczack et al 1976).

6 KININS AND MYOCARDIAL ISCHEMIA AND INFARCTION

In 1967 Sicuteri et al when determining plasma kininogen in 74 patients in the early phase of myocardial infarction found that plasma kininogen levels were decreased a phenomenon suggesting the activation of the plasma kinin system. In 1968 Wiegand et al determined plasma kininogen in 24 infarction patients and found that the kininogen level decreased being

4 KININS AND TISSUE INJURY

Tissue injury brings about the four cardinal signs characteristic of the early stages of inflammation - pain, vasodilation, increased vascular permeability and accumulation of leukocytes - which are all known actions of the kinins (Elliot et al 1960 Konzett and Stürmer 1960 Lewis 1962)

In addition there are two other points that suggest the role of kinins in the inflammatory process. First kinins together with other vasoactive substances have been detected at the site of local injury (Rocha e Silva and Antonio 1960 Rocha e Silva and Rosenthal 1961 Melmon et al 1967). Secondly there is evidence that proteolytic enzymes are activated during tissue injury (Beloff and Peters 1945 Ungar 1947). Moreover local conditions prevailing in inflamed tissue favor the formation and accumulation of the kinins. The lowering of pH at the site of injury might be a contributing factor in the activation of the kinin system (Merle 1934 Fray et al 1950). The change in pH of the interstitial space of inflamed tissue reduces the capacity of kininases to inactivate kinins (Edery and Lewis 1962).

Thus it seems possible that an inflammatory response to tissue injury of any kind could be brought about by kinin formation. Once initiated the inflammatory response might continue by virtue of continued kinin formation. Prekallikrein and possibly kininogen could simply diffuse from the blood vessels to the injured area or the active kallikrein could be brought to the site by leukocytes which are known to accumulate in the inflamed area (Lewis 1962).

It seems however unlikely that inflammation is mediated through a single chemical agent since various other substances e.g. histamine, serotonin and more recently prostaglandins (Willis 1969) have also been detected in the inflammatory area. These substances also exhibit actions characteristic of inflammatory response. So far the interrelationship between the different mediators in tissue injury induced inflammation is unclear.

Myocardial infarction may be considered an inflammatory response to anoxia - and on the basis of the present knowledge of biochemical pathology it can be assumed that the role which proteases play in inflammation is in general similar to that during myocardial infarction (Haberland 1975).

elevated and inhibitory activity as well as kininogen decreased. The changes in plasma prekallikrein and kallikrein inhibitory activity were associated with the severity of the infarction as judged by haemodynamic parameters.

Kimura et al (1975) studied 23 cases of acute myocardial infarction of which 16 were survival and 7 non survival. They determined plasma kininogen and bradykinin during the course of the disease. They noted a decrease with minimum level on the second day in kininogen with a simultaneous increase in bradykinin. They also found a significant correlation between the lowering of blood pressure and changes in the examined parameters. Furthermore they observed a significant correlation between plasma kininogen decrease, bradykinin increase and lowering of total peripheral resistance as well as increase of circulation time but not decrease of cardiac output. Unexpectedly they found that in the non-survival cases the bradykinin level was significantly lower than in the survival cases. They concluded that the release of kinins at the local myocardial site may perhaps be a beneficial compensatory mechanism contributing towards survival.

Golikov et al (1977) studied 88 patients with acute myocardial infarction with a fatal course and determined spontaneous BAEE-esterase (kallikrein) activity, prekallikrein and kallikrein inhibitory activity as well as epinephrine and norepinephrine levels in the blood. Spontaneous esterase activity was considerably increased and prekallikrein and kallikrein inhibitory activity decreased. Raised catecholamine levels were also observed within 6 hours after the onset of the disease.

Coronary artery ligation in dogs has been shown to cause a significant increase in bradykinin in parallel with a decrease in kininogen in the coronary sinus blood (Kimura et al 1973, Hashimoto et al 1977). These data support the hypothesis that bradykinin is produced and released from the site of myocardial injury.

Aprotinin, a serine esterase inhibitor capable of inhibiting kallikrein, has been used in experimental models of myocardial injury after coronary artery occlusion in the dog (see Wilkens et al 1975, Diaz et al 1977). The results indicate that aprotinin has beneficial effects, probably by changing the normal consequences of tissue damage (see Massion et al 1975). There are also reports of beneficial effects of aprotinin in clinical myocardial infarction (see Sicuteri et al 1975).

lowest 48 hours after the onset and rising thereafter. There was no difference in the kininogen levels of the patients in shock as compared with the other infarction patients. There was no correlation between the maximal serum GOT activity and the minimum of the kininogen level.

Dzizinski and Kulmov (1972) found when studying 70 acute myocardial infarction patients a decrease in plasma kininogen especially when complicated by shock. They noted also that in such patients the BAEe esterase (kallikrein) activity was characterized by a high variance of the individual values. The trypsin inhibitory activity indicating the degree of kallikrein inhibition was found to be increased in all patients with coronary insufficiency and highest in the acute phase of myocardial infarction.

Pitt et al (1970) observed that of 11 patients experiencing ischemia with angina or typical ST segment changes in electrocardiogram during pacing test seven showed activation of the plasma kallikrein system with increased spontaneous plasma TAME-esterase (kallikrein) activity. Using the same analytical methods Sicuteri et al (1972) found that there was no change in spontaneous TAME esterase activity in 16 infarction patients but prekallikrein was found significantly decreased with a minimum on the 8th day after the onset of the symptoms. In 1973 Kedra et al observed a decrease in plasma kininogen on the first and second days; the decrease was greater in patients with marked hemodynamic disturbances. No correlation was shown between the extensiveness of the infarction as judged on the basis of ECG changes and plasma kininogen level.

In the same laboratory another effort was made to evaluate the role of the plasma kinin system in infarction by determining prekallikrein kininogen and kininase in 54 infarction patients (Kolber-Postepska 1975). No correlation was observed between the extensiveness of the infarction and the examined parameters. Nor were there any differences in the changes in plasma prekallikrein between patients with hemodynamic disturbances and noncomplicated patients. Kininogen and prekallikrein were at the lowest simultaneously and were normalized after two weeks. Kininase activity in all patients was less than in the control and did not change in two weeks. The author concludes that the reduced kininase activity in patients with myocardial infarction may prolong the presence of kinins in plasma and thus increase their biological activity.

Gomazkov (1974) determined plasma spontaneous TAME-esterase (kallikrein) activity, prekallikrein and kallikrein inhibitory activity as well as plasma kininogen level in 20 patients with myocardial infarction. Prekallikrein was at its lowest on the second day and returned to normal by the fifth day. Spontaneous esterase activity was

MATERIAL

1 PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

The investigation was carried out on 65 patients with acute myocardial infarction admitted to Helsinki University Central Hospital in two periods of time during the year 1976

Patients over 75 years of age, alcoholics and patients who lived outside the Helsinki district were excluded. Those patients were also excluded who died within two days of their arrival and those who arrived more than 48 hours after the onset of the acute symptoms or could not give the exact time of the appearance of the symptoms. Otherwise the patients represent an unselected sample of acute myocardial infarction. The patients were followed throughout the stay in hospital, two weeks on the average, and were asked on a control visit one month from the onset of the symptoms.

The treatment of the patients was not influenced by the author and was in each case individually decided by the physician on duty. 46 patients made a control visit after one month. The age and sex distribution of the infarction patients is shown in Fig. 3.

All patients included belong to the first diagnostic category (Definite acute myocardial infarction) of the WHO classification (Ischaemic Heart Disease Registers. Report of the Fifth Working Group WHO Copenhagen 1971).

OBJECTS OF THE PRESENT STUDY

The participation of the plasma kinin system in hemodynamic response and in pain in myocardial infarction has been suggested. No clinical investigations so far published deal with all the essential components of the plasma kinin system. Attempts to correlate changes in various components with the clinical picture and parameters are only a few and show considerable discrepancy.

Thus it seemed desirable to obtain a more comprehensive view of the possible changes in the different components of the plasma kinin system and their relationship to one another during the evolution of the disease.

The aim of the study is to answer the following questions:

Does activation of the plasma kinin system occur in acute myocardial infarction as judged from the changes in plasma prekallikrein, kallikrein, kallikrein inhibitory activity, kininogen and kininase activity as compared with control patients and what is the time course of these changes?

Do the changes correlate with the extent and severity of the infarction as judged

by serum maximal creatine kinase (CK) activity

by comparing transmural with non transmural infarctions and

- by clinical hemodynamic criteria?

~ Do the changes in the plasma kinin system correlate with the duration of pain in acute myocardial infarction?

MATERIAL

1. PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

The investigation was carried out on 65 patients with acute myocardial infarction admitted to Helsinki University Central Hospital in two periods of time during the year 1976.

Patients over 75 years of age, alcoholics and patients who lived outside the Helsinki district were excluded. Those patients were also excluded who died within two days of their arrival and those who arrived more than 48 hours after the onset of the acute symptoms or could not give the exact time of the appearance of the symptoms. Otherwise the patients represent an unselected sample of acute myocardial infarction. The patients were followed throughout the stay in hospital, two weeks on the average, and were asked on a control visit one month from the onset of the symptoms.

The treatment of the patients was not influenced by the author and was in each case individually decided by the physician on duty. 46 patients made a control visit after one month. The age and sex distribution of the infarction patients is shown in Fig. 3.

All patients included belong to the first diagnostic category (Definite acute myocardial infarction) of the WHO classification (Ischaemic Heart Disease Registers Report of the Fifth Working Group WHO Copenhagen 1971).

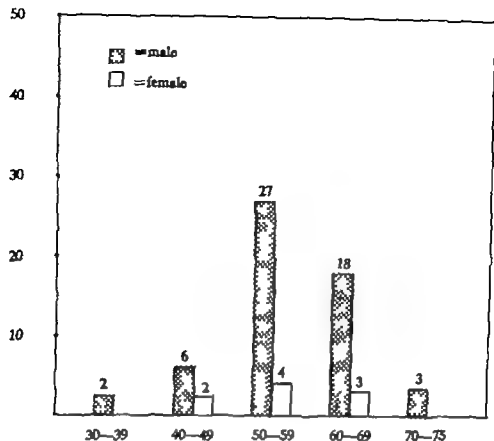


Fig 3 Age and sex distribution of the patients with acute myocardial infarction

II CONTROL PATIENTS

16 patients undergoing minor eye operations at the Clinic of Ophthalmology of Helsinki University Central Hospital constituted the control group. This group was followed for four days in the hospital, and they remained in recumbency during this time.

The possibility of silent myocardial infarctions in the control group was excluded by daily determinations of serum total creatine kinase activity.

The age and sex distribution of the whole patient material is shown in Table 1.

Table 1 Age and sex distribution of the patient material of the study

Group	Number of patients	Age, years mean (median) range	Sex
Control patients	16	56.0 (60.0) 28 75	M 10 F 6
Myocardial infarction (AMI)	65	55.8 (55.0) 35 72	M 59 F 9

METHODS

1 DEFINITION OF THE CLINICAL CONCEPTS

1 ACUTE MYOCARDIAL INFARCTION (AMI)

In this study the diagnosis of acute myocardial infarction was based on the clinical picture, electrocardiographic changes indicative of myocardial necrosis, and laboratory tests.

Appearance of pathologic Q-wave in successive ECG recordings together with ST segment and T-wave changes indicated transmural injury, whereas ST segment and T-wave changes alone were indicative of non-transmural (subendocardial) injury.

All diagnoses were primarily made by the physicians in charge of the patients. In all patients the serum total CK activity was determined on the first three days after admission. Since it is often difficult to distinguish prolonged anginal attacks from mild forms of infarction, the final differentiation of these borderline cases was based on detection of myocardial specific (Sobel et al. 1972; Kontinen and Somer 1973) MB-isoenzyme of CK in serum during the first three days after admission.

2 HEART FAILURE

Heart failure is defined as a condition in which the heart is unable to maintain an adequate cardiac output in relation to venous return or the needs of the tissues. Acute heart failure may be subdivided into acute congestive heart failure and shock.

by signs of venous congestion and venous hypertension behind the left ventricle. In cardiogenic shock the clinical picture is characterized by severe systemic arterial hypotension with signs of inadequate tissue perfusion. In acute myocardial infarction heart failure frequently has the characteristics of congestive heart failure and shock simultaneously.

The bedside signs suggestive of congestive heart failure of left ventricular origin were as follows: dyspnea at rest, orthopnea, tachypnea, attacks of wheezing in the absence of pulmonary disease, rapid forced respiration and moist rales over the lung fields and prolonged sinus tachycardia of over 110 beats per minute. Patients were considered to have clinical pulmonary congestion only if there was both radiographic and auscultatory evidence.

Clinical signs of congestive heart failure of right ventricular origin were as follows: enlarged, often tender liver, peripheral sacral and ankle edema without a local cause and rise of jugular venous pressure when it was clearly observable.

The grade of peripheral hypoperfusion was assessed by the presence of decreased temperature of the skin, pallor, cyanosis, restlessness, confusion, decreased urinary output and rapid feeble pulse with systemic arterial hypotension of 100 mmHg or below.

In this study the patients were classified into three rigorously defined subgroups according to clinical hemodynamic criteria. The subdivision was performed by an expert cardiologist according to clinical records which in each case had been completed bedside by the author.

II CLINICAL EXAMINATION OF THE PATIENTS AND SAMPLE COLLECTION

The patients included in the present series were examined by the author on the same days as the blood samples were taken for the various analyses of the study. In the beginning of the follow-up a history of earlier cardiovascular and other diseases was taken and the onset of the present symptoms was carefully recorded. In clinical examination particular attention was paid to various manifestations such as paradoxical pulsation, pericardial friction rub and atrial and ventricular gallops which may give diagnostic support in suspected myocardial infarction. In addition careful examinations were performed to detect signs of congestive heart failure and/or signs of decreased peripheral perfusion as described in the preceding chapter.

Conventional 12 lead electrocardiograms were taken of all patients during the first days after admission. The routine laboratory tests depended on whether the patient was admitted to the intensive care unit or transferred to an ordinary ward. Anteroposterior roentgenograms were taken daily of the patients in the intensive care unit. Moreover heart function was monitored and central venous pressure and/or pulmonary wedge pressure measured during the stay in the unit.

The first blood sample was taken on admission and the subsequent samples on the 2nd 3rd 4th and 6th 8th days after the onset of the symptoms. An additional sample was taken after one month on a control visit. Of the control patients samples were taken on admission the second day and on the third or the fourth day in hospital.

For the assay of the components of the kallikrein system citrated plasma was prepared as follows. 9 ml of venous blood from antecubital fossa was taken with a siliconized needle into a plastic tube containing 1 ml of 3.8 % sodium citrate and mixed. After centrifugation at 2000g for 15 minutes at room temperature the plasma samples were frozen at -20°C in several aliquots.

For the assay of kininogen and kininases an additional sample of 10 ml of venous blood was taken through the same needle into a plastic tube containing 500 units of heparin (Heparin Medica) mixed and centrifuged. The plasma was prepared as described later for the assay of kininogen and kininases.

Serum total protein and serum total creatine kinase activity and IB isoenzyme activity determinations were carried out on serum samples taken through the same needle.

The samples in the intensive care unit were taken through a central venous catheter through which 10 ml of blood had been passed before collecting. There were no differences in the measured components as ascertained by several samples taken by both means whether the samples were collected via CVP catheter or from the brachial veins.

It is also ascertained that freezing of the plasma samples once did not destroy the activities measured when the final assays were carried out within 5 months from the collection of the samples.

III: METHODS OF ASSAY

The activation of the plasma kinin system should lead to consumption and consequent decrease of plasma prekallikrein and kininogen with a

by signs of venous congestion and venous hypertension behind the left ventricle. In cardiogenic shock the clinical picture is characterized by severe systemic arterial hypotension with signs of inadequate tissue perfusion. In acute myocardial infarction heart failure frequently has the characteristics of congestive heart failure and shock simultaneously.

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also hydrolyzed but more slowly the rates decreasing in the above order (Webster and Pierce 1961)

Mainly BAEe and TAME have been used as substrates in kallikrein assays. The assays based on BAEe-hydrolysis all use BAEe in a final concentration of about 1 mM which is far below the K_m of plasma kallikrein reported at 13.6 mM (Colman et al 1971) and therefore significantly limits the accuracy of these assays. Consequently of the esterolytic methods that of Colman et al (1969) using TAME has proved to be most useful especially since it allows simultaneous measurement of spontaneous esterase (kallikrein) prekallikrein and kallikrein inhibitory activity.

The assay is based on the observations that citrated normal human plasma collected without exposure to glass contains extremely low levels of spontaneous arginine esterase activity. The increase of esterase activity after exposure to kaolin results primarily from the activation of plasma prekallikrein and that the rapid fall in arginine esterase activity after maximal activation is due to plasma kallikrein inhibitors (Colman et al 1969 b).

The substrate used is N-tolylsulfonyl L-arginine-methylester (TAME). Its hydrolysis liberates methanol which is measured according to the original method of Siegelman et al (1962). The time course of the activation by kaolin is typical. A maximum in normal plasma is obtained in 1-2 minutes with a decline thereafter to a level which remains the same for several minutes (Fig. 4).

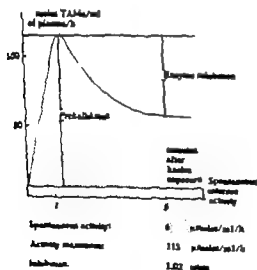


Fig. 4 The activation curve of the plasma kallikrein system. Control from the mean of 48 measurements of 16 normal patients. The curve represents the plasma esterase activity at different time intervals after kaolin activation at 2 min.

concomitant rise in plasma kallikrein and bradykinin. Changes are also expected in kallikrein inhibitory as well as kininase activities.

Demonstration of elevated free bradykinin alone certainly suggests its participation in a pathological process but hardly gives a reliable picture of the state of the plasma kinin system at a given moment. In addition, measurement of plasma free bradykinin has so far appeared extremely unreliable because of the short biological half life of the peptide and because of the problems of specificity and relative insensitivity of the assay methods available.

Both bioassay and radioimmunoassay have been used in measuring plasma free bradykinin (see Trautsohold 1970). A common problem for both is the difficulty in the sample collection due to the instability of bradykinin. Bioassay is unreliable because of the potentiating factors in plasma (Louhija and Sipilä 1972) and it usually lacks the necessary sensitivity. The unreliability of the methods for free bradykinin determination is evident from the fact that various authors have reported normal plasma bradykinin levels varying from 0.2 ng/ml (Zacest and Mashford 1967; Mashford and Roberts 1972) to 3 ng/ml (Talamo et al 1969).

With the radioimmunoassay for bradykinin developed in this laboratory (range of measurement 1-20 ng BK/sample) no free bradykinin activity in normal human plasma could be detected. Thus plasma free bradykinin measurement was not attempted in this study but evidence of possible activation of the plasma kinin system was sought by measuring those components which are more stable and for which there exist more reliable methods of assay.

1 PLASMA KALLIKREIN, PREKALLIKREIN AND KALLIKREIN INHIBITORY ACTIVITY

Plasma kallikrein (EC 3.4.21.8) has been assayed mainly by two approaches by means of bioassay which measures the kinin released by the proteolytic action of kallikrein and by chemical methods based on the esterolytic properties of kallikrein. Because of the known disadvantages of bioassay chemical methods using synthetic basic amino acid esters, notably arginine derivatives as substrate, have been more widely preferred. Of the synthetic esters commercially available, all kallikreins hydrolyze N-benzoyl-L-arginine-ethylester (BAEE) most effectively. N-benzoyl-L-arginine-methylester (BAME) (Webster and Pierce 1961), N-tolylsulfonyl-L-arginine-methylester (TAME) and N-acetyl-L-tyrosine-ethylester (ATEE) are

COMMENTS

The method is not specific for free kallikrein since a number of other proteolytic enzymes e.g. plasmin, trypsin, thrombin and C1 esterase will also hydrolyse TAME (Colman et al. 1969). When elevated spontaneous esterase activity is encountered a further characterization of the enzyme profile is necessary.

For plasma prekallikrein this method is reasonably specific since it has been shown that rising esterase activity after kaolin activation is due to plasma prekallikrein (Colman et al. 1969 b).

This method also allows the measurement of total kallikrein inhibitory activity of plasma. Activated plasma kallikrein has been shown to bind to alpha 2-macroglobulin (Harpel 1970). Since the complex bound kallikrein can still be measured with synthetic ester substrates (Barrett and Starkey 1973) possibly bound activity is included in the present measurements.

Since the activation curve rises very rapidly after kaolin exposure (Fig. 4) it is important to try to come as close to the activity maximum as possible. In this laboratory it was found that especially with pathological plasma the kaolin activation time may be significantly delayed and thus with only one sample taken e.g. at 1 minute as suggested by Colman et al. the maximum may be missed. Therefore we made activity determinations at 1 minute, 1.5 minutes and 2 minutes.

The normal values obtained (Fig. 4) agree well with the normal values of Colman et al. (1969): spontaneous esterase activity 7 μ moles/ml/h, activity at one minute (maximum) 97 μ moles/ml/h and inhibitory activity 1.00 IU.

2. PLASMA KININOGEN

Plasma kininogen has usually been measured by bioassay of bradykinin totally liberated by trypsin from heat acid denatured plasma (Diaz and Carvalho 1963). Along with the usual disadvantages of bioassay the most serious drawback of this method is the possible involvement of bradykinin potentiating factors (Hamberg 1968, Aarsen 1968, Louhija and Sipilä 1971).

A method for kininogen determination based on radioimmunoassay of trypsin liberated bradykinin has recently been introduced (Sipilä and Louhija 1976). This method is more suitable for serial determinations than bioassay and it gives results that are in good agreement with those

which represents the spontaneous esterase (kallikrein) activity. Further samples were taken at 1, 1.5, 2 and 5 minutes. The peak value minus spontaneous activity represents total prekallikrein and enzyme inhibition can be calculated from the value after levelling off.

Marked glass tubes each containing 41.9 mg of TAME (N^a Tosyl L-arginin-methylesterhydrochlorid Merck) in 2 ml of 0.1 M sodium phosphate buffer pH 7.6 in 0.15 M NaCl were placed in an ice bath.

For the measurement of spontaneous arginine esterase (kallikrein) activity 0.1 ml of the first test sample and 0.1 ml of the buffer were added with plastic pipette to the first TAME tube in the series. For the measurement of prekallikrein and kallikrein inhibitory activity equal volumes of kaolin (Kaolin Baker) suspension (10 mg/ml) in buffer and of test sample were mixed in plastic tubes and incubated at 25°C in a water bath. At the end of 1 minute, 1.5 minutes, 2 minutes and 5 minutes aliquots of 0.2 ml were pipetted over and immediately mixed in respective TAME tubes. The tubes were then withdrawn from the ice bath and placed in a water bath at 37°C. At 1 minute and at 31 minutes aliquots of 1 ml were removed from each tube and added to 0.5 ml of 15% trichloroacetic acid and centrifuged.

0.5 ml of the supernatant was removed and placed in a separate test tube containing 0.1 ml of 2% potassium permanganate. After mixing vigorously for 1 minute 0.1 ml of 10% sodium sulfite was added and mixed. 4 ml of 0.2% chromotropic acid in 65% sulfuric acid was added and mixed thoroughly. The samples were placed in a boiling water bath for 15 minutes and thereafter allowed to cool.

Each hydrolyzed (31 minutes) sample was measured against its own nonhydrolyzed (1 minute) blank set at zero at 580 m μ in a Beckman DU spectrophotometer at 25°C. Using weighed amounts of methanol as standards it was found that an absorbance of 0.100 is equivalent to 36.5 μ moles of methanol released or TAME hydrolyzed per milliliter of plasma per hour.

Spontaneous esterase activity and prekallikrein are expressed as μ moles of TAME hydrolyzed/ml of plasma/hour. The kallikrein inhibitory activity can be expressed as follows: 1 U of kallikrein inhibition is that amount of inhibitory activity that at 5 minutes gives 50% inhibition of the maximum value. The number of units was computed from a calibration plot of $\log \frac{\text{activity at 5 minutes}}{\text{activity maximum}}$ vs. inhibitory units. The standard deviation of the mean of 14 prekallikrein measurements obtained from the same sample was 1.9 %.

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Bradykinin was liberated from plasma kininogen according to the method of Diniz et al (1961) by trypsin incubation of the heat acid denatured plasma samples. Bradykinin immunogen was prepared by coupling bradykinin to human serum albumine. The radioimmunoassay procedure was a slight modification of that of Talamo et al (1968)

0.2 ml of heparin plasma was added to 1.8 ml of 0.2% acetic acid in plastic tubes and heated in boiling water bath for 30 minutes. The pH of the mixture was adjusted to 7.4-7.8 by adding 0.1 N NaOH and buffered to pH 7.8 at 37°C (Astrup pH-meter Radiometer Copenhagen) by adding 0.5 ml of 0.2 M Tris buffer. 200 µg of crystallized trypsin in 0.1 ml of saline was added and the mixture was incubated for 30 minutes at 37°C in a water bath. The incubation was stopped by adding 5 ml of boiling ethanol and the mixture was further incubated for 10 minutes at 70°C. The mixture was cooled, centrifuged and the supernatant taken to dryness in a siliconized 100 ml round bottomed flask under reduced pressure (Rotavapor Buchi) and at a temperature of 35°C. The dried precipitate was stored at 20°C until assay. For the assay the precipitate was dissolved in 2 ml of veronal acetate saline-buffer with 1% gelatine which is the best stabilizer of weak bradykinin solutions.

Bradykinin immunogen was prepared by coupling bradykinin to human serum albumin using water-soluble carbo-di-imide. 12.5 mg of human serum albumin (Kabi) was dissolved in 0.5 ml of distilled water. 30 mg of bradykinin triacetate (Sigma) was added and dissolved and followed by 450 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbo-di-imide HCl (Ott Chemical Company). The solution was mixed kept in ice bath for 48 hours at +4°C. 4 ml of distilled water was added and the solution was thoroughly mixed with 4 ml of Freund's complete adjuvant (Difco). The immunogen was injected intramuscularly into all four legs of the rabbits to be immunized. One rabbit out of fifteen produced a usable antiserum. The antiserum was used in a dilution of 1:100. Using this solution the ratio of the radioactivity bound to antibody in absence of unlabelled bradykinin was about 40%.

A radio-labelled tracer was prepared according to Greenwood et al (1963). 20 µl of 0.5 M phosphate buffer pH 7.0, 50 µl of tyrosine-bradykinin solution (0.5 mg/ml) (New England Nuclear) and 25 µl of Chloramine-T (5 mg/ml) (Merck) were added to about 2 mCi (125 I) sodium iodide (IMS 30 The Radiochemical Centre, Amersham). After 45 seconds 100 µl of sodium metabisulfite (2.5 mg/ml) (Merck) was added and immediately thereafter 5 µl of potassium iodide solution (20 mg/ml in 0.1 N acetic acid) (Merck). The solution was purified from unbound iodine by chromatography on a 4 ml Dowex column (Dowex 1x8 Cl⁻ 200-400 mesh) (Fluka) in a disposable plastic pipette. The elution was made using water. The fraction (0.5 ml) containing the top activity of bound iodine was diluted to get a working solution of 2000 counts per minute per 5 µl.

The radioimmunoassay was carried out at room temperature as follows. Throughout the procedure all solutions contained 1% of gelatine. 20 µl of unknown or standard bradykinin solution (bradykinin triacetate Sigma) 5 µl of (125 I) tyr⁰ bradykinin working solution and 5 µl of 1:100 dilution of antiserum all in veronal acetate buffered saline pH 7.4 (veronal 1.47 mg/ml and acetate 9.7 mg/ml in 0.13 M saline) were added in this order into 1 ml conical plastic centrifuge tubes (Fischer). The amount of

non-specific binding of bradykinin to rabbit serum was controlled by including a tube of non immunized rabbit serum to each analysis. The incubation was carried out at room temperature for 1 hour which was found to be enough for optimal binding. At the end of incubation period 400 μ l of dextran coated charcoal suspension was added. The mixture was allowed to stand for 10 minutes and was centrifuged and 100 μ l of the supernatant was counted for radioactivity. A standard curve was plotted from 6 standard samples each time and the amount of bradykinin in duplicate unknown samples was derived from this curve.

The range of the assay lies between 1 and 20 ng of bradykinin in the sample. The standard deviation of the differences from the mean of 12 measurements obtained from the same sample was $2 \pm \%$ at the level of 65 % inhibition and 3.0 % at the level of 45 % inhibition. The interassay standard deviation was 7 % derived from 28 duplicate determinations.

COMMENTS

With this method the mean kininogen content in plasma of 26 normal individuals was 3.36 ± 0.096 (S.E.M.) μ g of bradykinin/ml of plasma which is in agreement with the results of Hamberg (1969) 4.31 ± 0.067 (S.E.M.) μ g of bradykinin/ml of plasma for total plasma kininogen of pooled human blood bank plasma using bioassay on the isolated guinea pig.

In the present study the results for plasma kininogen are expressed as μ g of bradykinin/ml of plasma and μ g of bradykinin/g of serum protein in order to eliminate the effects of any hemoconcentration or hemodilution (see Haberman 1970).

3. PLASMA KININASES

In this study plasma kininase (EC 3.4.12.7 EC 3.4.15.1) activity was determined by a method based on radioimmunoassay for bradykinin (Sipilä 1977).

Heparin plasma was diluted 10 to 1 by 0.05 M phosphate buffer pH 7.5. In a small plastic tube 0.9 ml of this solution was mixed with 500 ng of synthetic bradykinin (Bradykinin triacetate Sigma) in 0.1 ml of phosphate buffer. Immediately after mixing the tube was incubated at 37°C in a water bath. Samples of 0.1 ml were taken after 2 minutes, 6 minutes and 10 minutes incubation. Samples were immediately mixed with an equal volume of phosphate buffer in plastic tubes kept in ice. The phosphate buffer in these tubes contained 0.003 M of disodium EDTA to stop the kininase activity.

obtained with bioassay after elimination of bradykinin potentiating factors (Louhijä and Sipilä 1972)

Bradykinin was liberated from plasma kininogen according to the method of Diniz et al (1961) by trypsin incubation of the heat acid denatured plasma samples. Bradykinin immunogen was prepared by coupling bradykinin to human serum albumine. The radioimmunoassay procedure was a slight modification of that of Talamo et al (1968)

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Bradykinin immunogen was prepared by coupling bradykinin to human serum albumin using water-soluble carbo-di-imide. 12.5 mg of human serum albumin (Kabi) was dissolved in 0.5 ml of distilled water. 30 mg of bradykinin triacetate (Sigma) was added and dissolved and followed by 450 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbo-di-imide HCl (Ott Chemical Company). The solution was mixed, kept in ice bath for 48 hours at +4°C. 4 ml of distilled water was added and the solution was thoroughly mixed with 4 ml of Freund's complete adjuvant (Difco). The immunogen was injected intramuscularly into all four legs of the rabbits to be immunized. One rabbit out of fifteen produced a usable antiserum. The antiserum was used in a dilution of 1:100. Using this solution the ratio of the radioactivity bound to antibody in absence of unlabelled bradykinin was about 40%.

A radio-labelled tracer was prepared according to Greenwood et al (1963). 20 µl of 0.5 M phosphate buffer pH 7.0, 50 µl of tyrosine-bradykinin solution (0.5 mg/ml) (New England Nuclear) and 25 µl of Chloramine-T (5 mg/ml) (Merck) were added to about 2 µCi (125 I) sodium iodide (IMS 30 The Radiochemical Centre Amersham). After 45 seconds 100 µl of sodium metabisulfite (2.5 mg/ml) (Merck) was added and immediately thereafter 5 µl of potassium iodide solution (20 mg/ml in 2 N acetic acid) (Merck). The solution was purified from unbound iodine by chromatography on a 4 ml Dowex column (Dowex 1x8 C1 200-400 mesh) (Fluka) in a disposable plastic pipette. The elution was made using water. The fraction (0.5 ml) containing the top activity of bound iodine was diluted to get a working solution of 2000 counts per minute per 5 µl.

The radioimmunoassay was carried out at room temperature as follows. Throughout the procedure all solutions contained 1% of gelatine. 20 µl of unknown or standard bradykinin solution (bradykinin triacetate Sigma), 5 µl of (125 I) tyr⁸ bradykinin working solution and 5 µl of 1:100 dilution of antiserum all in veronal acetate buffered saline pH 7.4 (veronal 1.47 mg/ml and acetate 9.7 mg/ml in 0.13 M saline) were added in this order into 1-2 ml conical plastic centrifuge tubes (Fischer). The amount of

IV STATISTICS

Conventional statistical methods were used for the calculations of mean values and of S.E.M. The significance of difference between sample means was estimated by the two tailed student's t test and probabilities above 0.05 were considered non-significant. Regression analysis of linear correlations was carried out according to the method of least squares.

promptly (Yang and Erdős 1967) and 1 % of gelatine to preserve the bradykinin activity. Unspecific binding of diluted plasma in the radioimmunoassay was monitored by adding to the analysis a control tube with mixture of diluted plasma and EDTA-gelatine phosphate buffer in equal amounts.

The results were plotted on a graph and the activity of kininases was expressed in IU. One mIU of kininase activity inactivates 1.06 µg of bradykinin per minute (Trautschold 1970). The standard deviation from 8 duplicate measurements of the same sample was 2.1 %.

COMMENTS

Methods in which plasma is incubated with added amounts of synthetic bradykinin are not specific for a particular kininase. The present method thus measures the total kininase activity in plasma.

4. SERUM TOTAL CREATINE KINASE (CK) AND MB ISOENZYME

Serum total CK activity (EC 2.7.3.2) was determined from deep-frozen samples with Boehringer CPK test sets (Boehringer Mannheim, West Germany) at 25°C. The activity is expressed as IU/l. The upper normal value is 50 IU/l.

The MB isoenzyme activity was determined by the method of Sober and Kontinen (1972). Since the MB isoenzyme was used as a plus-or-minus test for detection of myocardial damage no quantification was performed.

5. SERUM TOTAL PROTEIN

Serum total protein was determined by the biuret method (Weichselbaum 1946) from deep-frozen serum samples.

IV STATISTICS

Conventional statistical methods were used for the calculations of mean values and of S.E.M. The significance of difference between sample means was estimated by the two tailed student's t test and probabilities above 0.05 were considered non-significant. Regression analysis of linear correlations was carried out according to the method of least squares.

RESULTS AND DISCUSSIONS

I THE COMPONENTS OF THE PLASMA KININ SYSTEM IN ACUTE MYOCARDIAL INFARCTION

I PLASMA KALLIKREIN PREKALLIKREIN AND KALLIKREIN INHIBITORY ACTIVITY

Table II shows the spontaneous TAME-hydrolyzing (kallikrein) activity of citrated plasma in acute myocardial infarction and control groups. There are no significant differences between the two groups and no significant changes during the follow up.

Table II Spontaneous esterase activity (μ molesTAME/ml of plasma/h) in control and acute myocardial infarction (AMI) (means and S.E.M.)

Group (N)	<24 h	24-48 h	48-96 h	8 d	30 d
Control (16)	5.3 ± 0.23	5.8 ± 0.24	6.1 ± 0.24	—	—
AMI (65)	6.2 ± 0.22	6.1 ± 0.21	6.1 ± 0.21	6.2 ± 0.22	6.3 ± 0.27

Fig. 5 presents plasma prekallikrein in acute myocardial infarction and control groups. There are no changes in the control group during the follow-up and the initial value of the infarction group does not differ from the control values. In the infarction group plasma prekallikrein decreases after 24 hours from the beginning of the symptoms, being at its lowest on the 3rd-4th day of the disease and rising thereafter. The difference is statistically significant on the second day ($t = 2.602$, $df = 79$, $p < 0.01$), third to fourth day ($t = 3.873$, $df = 77$, $p < 0.001$) as compared with the respective control values, and after a week ($t = 4.791$, $df = 123$, $p < 0.001$) as compared with the initial value of the infarction.

group as there are no control values at one week

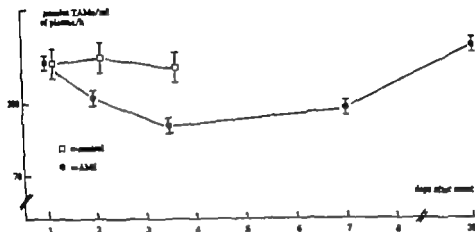


Fig 5 Plasma prekallikrein (mean and S.E.M.) in control and acute myocardial infarction (AMI)

The kallikrein inhibitory activity is initially higher in the infarction group than in the control group. The difference is statistically significant on the first day of the disease ($t = 1.975$, $df = 77$, $p < 0.05$). The results are presented in Table III.

Table III Kallikrein inhibitory activity (inhibitory units) in control and acute myocardial infarction (AMI) (mean and S.E.M.)

Group (N)	<24 h	24-48 h	48-96 h	6-8 d	30 d
Control (16)	1.02 ± 0.029	1.01 ± 0.034	1.03 ± 0.044	—	—
AMI (65)	1.14 ± 0.028	1.06 ± 0.025	1.01 ± 0.024	1.03 ± 0.024	1.09 ± 0.026

RESULTS AND DISCUSSIONS

I THE COMPONENTS OF THE PLASMA KININ SYSTEM IN ACUTE MYOCARDIAL INFARCTION

1 PLASMA KALLIKREIN PREKALLIKREIN AND KALLIKREIN INHIBITORY ACTIVITY

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Fig. 5 presents plasma prekallikrein in acute myocardial infarction and control groups. There are no changes in the control group during the follow-up and the initial value of the infarction group does not differ from the control values. In the infarction group plasma prekallikrein decreases after 24 hours from the beginning of the symptoms, being at its lowest on the 3rd-4th day of the disease and rising thereafter. The difference is statistically significant on the second day ($t = 2.602$, $df 79$, $p < 0.01$), third to fourth day ($t = 3.873$, $df 77$, $p < 0.001$), as compared with the respective control values, and after a week ($t = 4.791$, $df 123$, $p < 0.001$) as compared with the initial value of the infarction.

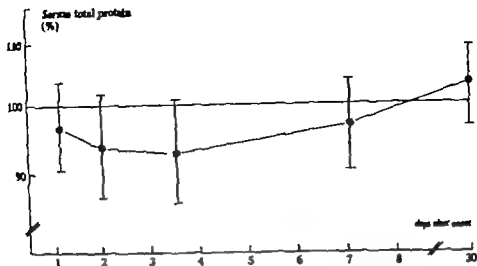


Fig 8 Serum total protein in the 65 infarction patients of the study (mean \pm SD). The 100% line represents the control level calculated from 48 measurements of the 18 control patients serum protein value. One or two stars indicate statistical significance at the 0.05 or 0.01 probability level respectively.

It can be seen that kininogen reaches its minimum after 48 hours from the onset and returns to the control level in one week. The statistical significances are as follows: Kininogen (expressed as μg bradykinin/ml of plasma) as compared with the control: on the first day $t = 2.075$ $df = 77$ $p < 0.05$; on the second day $t = 2.910$ $df = 78$ $p < 0.01$; on the third to fourth day $t = 3.211$ $df = 77$ $p < 0.001$. Kininogen (expressed as μg bradykinin/g of serum protein) as compared with the control: on the first day $t = 1.650$ $df = 77$ $p < 0.05$; on the second day $t = 1.819$ $df = 78$ $p < 0.05$; on the third to fourth day $t = 1.896$ $df = 77$ $p < 0.05$.

Fig 9 shows the plasma kininase activity in 19 infarction patients at the time when plasma kininogen was lowest and at the time of recovery compared with the values of 10 control patients. There are no differences between the groups.

DISCUSSION

In previous reports both unchanged (Dzizinski and Kulakov 1972; Siciteri et al 1972) and elevated (Gomazkov 1974; Golikov et al 1977) spontaneous esterase (kallikrein) activities in myocardial infarction have been observed. There are several factors that could explain the differing results. It is possible that changes in plasma free

2 PLASMA KININOGEN AND KININASES

Plasma kininogen expressed as μg of bradykinin/ml of plasma is shown in Fig 6 and as μg of bradykinin/g of serum protein in Fig 7. It was observed that serum total protein decreases significantly during the course of the disease being lowest on the third to fourth day (Fig 8)

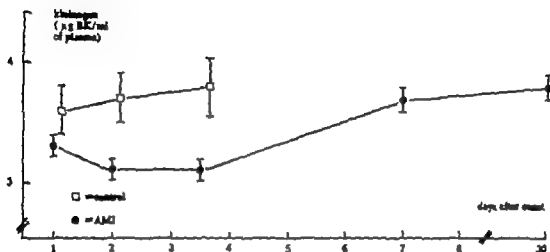


Fig 6 Plasma kininogen (expressed as μg BK/ml of plasma) in control and acute myocardial infarction (AMI) (means and S.E.M.)

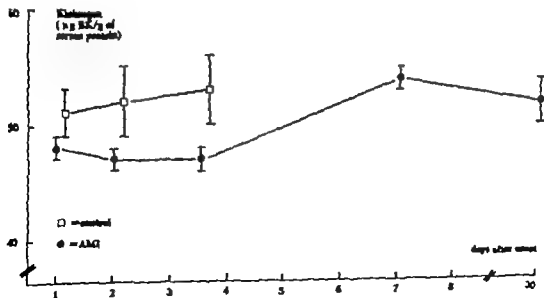


Fig 7 Plasma kininogen (expressed as μg BK/g of serum protein) in control and acute myocardial infarction (AMI) (means and S.E.M.)

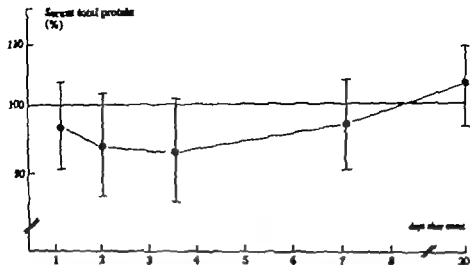


Fig 8 Serum total protein in the 65 infarction patients of the study (means and S D). The 100% line represents the control level calculated from 46 measurements of the 18 control patients' serum protein values. One or two stars indicate statistical significance at the 0.05 or 0.01 probability level respectively.

It can be seen that kininogen reaches its minimum after 48 hours from the onset and returns to the control level in one week. The statistical significances are as follows: Kininogen (expressed as μg bradykinin/ml of plasma) as compared with the control: on the first day $t = 2.075$, $df = 77$, $p < 0.05$; on the second day $t = 2.910$, $df = 78$, $p < 0.01$; on the third to fourth day $t = 3.211$, $df = 77$, $p < 0.001$. Kininogen (expressed as μg bradykinin/g of serum protein) as compared with the control: on the first day $t = 1.650$, $df = 77$, $p < 0.05$; on the second day $t = 1.819$, $df = 78$, $p < 0.05$; on the third to fourth day $t = 1.896$, $df = 77$, $p < 0.05$.

Fig 9 shows the plasma kininase activity in 79 infarction patients at the time when plasma kininogen was lowest and at the time of recovery compared with the values of 10 control patients. There are no differences between the groups.

DISCUSSION

In previous reports both unchanged (Ozizinski and Kulmov 1972; Sicuteri et al 1972) and elevated (Gomazkov 1974; Golikov et al 1977) spontaneous esterase (kallikrein) activities in myocardial infarction have been observed. There are several factors that could explain the differing results. It is possible that changes in plasma free

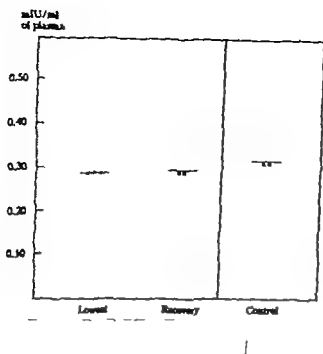


Fig 9 Plasma kinase activity in acute myocardial infarction on the day when plasma kininogen level was lowest and at the time of recovery compared with the control group

kallikrein take place very early and are only transient because of the continuously acting plasma kallikrein inhibitors. It is also possible that changes are not observed owing to the relative insensitivity of the method. On the other hand elevated esterase activity may also be due to other proteolytic enzymes in plasma.

The observation of augmented kallikrein inhibitory activity during the very early stage of the disease may indicate an actual triggering of the activation of the plasma kinin system. After the increase there is a gradual decrease. Dzizinski and Kufimov (1972) observed markedly elevated trypsin inhibitory activity in acute myocardial infarction. Sicuteri et al (1975) noticed a gradual decrease in kallikrein inhibitor using the same method as has been used in this study. The decrease paralleled the lowering of prekallikrein, the maximum of the loss appearing on the 8th to 10th day of the disease.

It is possible that the results obtained by Sicuteri et al (1975) (i.e. the later appearance of the maximal prekallikrein decrease as compared with the present results as well as the decrease in kallikrein inhibitory activity) is due to a methodological difference. It was observed in this laboratory that with pathological plasma the activation time after kaolin exposure may be delayed beyond one minute. Thus if the one minute sample is taken to represent prekallikrein the values will be too low. Consequently since the five minute value is constant the inhibitory activity may also be misinterpreted.

Plasma kininogen reaches its minimum after 48 hours and it is also normalized sooner than prekallikrein i.e. in one week. This is in

good agreement with previous reports by others. Since serum protein also decreases during the disease it is necessary to correct the kininogen values in proportion to serum protein. It can be seen that the changes in plasma kininogen still remain significant after this correction (Fig 7.)

According to the present results there are no changes in plasma kininase activity during acute myocardial infarction. No attempt was made in this study to activate possible latent kininases in plasma. In addition it should be remembered that not only plasma but also the tissues especially the lungs are potential sites of kinin inactivation. Kolber-Postepska (1975) observed a decrease in plasma kininase activity in patients with acute myocardial infarction. The activity did not increase towards the recovery.

II PLASMA PREKALLIKREIN AND KININOGEN AS RELATED TO MAXIMAL SERUM TOTAL CK ACTIVITY

Serum total CK activity was determined of all the patients in the infarction group on the first three days after admission. Peak value of serum total CK appears within 16 to 30 hours from the onset of acute symptoms (Sobel et al 1972). Maximal serum total CK value within these time limits was taken to represent each patient's total CK maximum which was then compared with the maximal prekallikrein and kininogen decrease. The results are shown in Fig 10 and Fig 11. The correlation is significant in both.

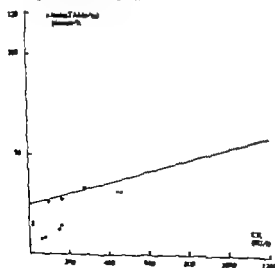


Fig 10 Relation between maximal plasma prekallikrein decrease and maximal serum total CK activity in acute myocardial infarction ($r = 0.4261$, $t = 3.4722$, $p < 0.01$, $N = 38$)

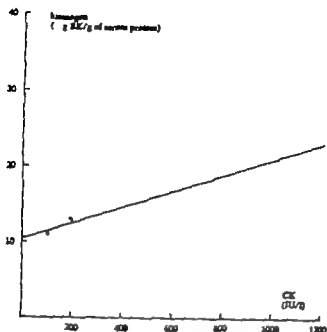


Fig 11 Relation between maximal plasma kininogen decrease (expressed in proportion to serum total protein) and maximal serum total CK activity in acute myocardial infarction
 $y = 10.48 + 0.011x$ $r = 0.4365$ $t = 2.829$ $p < 0.01$
 $N = 36$

DISCUSSION

It is generally agreed that certain serum enzyme levels after myocardial infarction roughly correlate with infarct size (Mydick et al 1955 see Sobel et al 1972). In this study maximal serum total creatine kinase was compared with the changes in plasma prekallikrein and kininogen.

The mean appearance time of the peak value of serum total CK activity in the group was 23.7 hours, which is in good agreement with two other reports (Mathey et al 1974, Inoue et al 1977) 24 hours both. The mean maximal total CK value at this time 369 IU/l is also in agreement with the results of other authors (Mathey et al 1974) 380 IU/l.

Because intramuscular injections can cause a release of extracardiac CK (Helzer et al 1970, Zoner and Harrison 1974, Klein et al 1973), all patients who received intramuscular injections were excluded from CK-grouping. For the same reason patients who had been resuscitated or defibrillated were also excluded (Konttinen et al 1969). The most severe hemodynamic group, including 7 patients, was also excluded since extracardiac CK release has also been reported in shock (Klein et al 1973).

The present results suggest that there is a correlation between the infarct size and the decrease in plasma prekallikrein and kininogen. There is only one previous attempt in the literature to correlate serum enzyme changes with the plasma kinin system (Wiegandhausen et al 1968). No correlation was found between the maximal serum LDH-activity and the minimum plasma kininogen level.

III PLASMA KALLIKREIN INHIBITORY ACTIVITY PREKALLIKREIN AND KININOGEN IN PATIENTS WITH TRANSURAL VS NON-TRANSURAL INFARCTION

52 patients had transmural and 13 had non-transmural infarction as judged by ECG.

Kallikrein inhibitory activity was significantly elevated in the first 24 hours of the disease in the patients with transmural infarction as compared with the non-transmural infarction patients.

Maximal plasma prekallikrein decrease takes place at the same time in both infarction groups but is greater in patients with transmural infarction.

Maximal plasma kininogen decrease expressed in proportion to serum total protein takes place later in the transmural infarction group than in the non-transmural group. It is significantly greater in the transmural group than in the non-transmural group. The results are presented in Table IV.

Table IV Plasma kallikrein inhibitory activity (IU) on the first day of the disease and maximal plasma prekallikrein decrease (nmoles IAPU/ml of plasma/h) and maximal plasma kininogen decrease (μ g B₁/g of serum protein) at the time of their appearance (mean) (transmural and non-transmural infarction patients (means and S.E.M.))

	Transmural (N 52) (time of appearance)	Non-transmural (N 13) (time of appearance)	p
Kallikrein inhibitory activity	1.17 0.044 (24 h)	1.02 0.014 (24 h)	<0.05
Maximal prekallikrein decrease	37.7 0.36 (60 h)	24.4 1.21 (60 h)	<0.05
Maximal kininogen decrease	15.0 0.13 (40 h)	10.7 0.17 (36 h)	<0.05

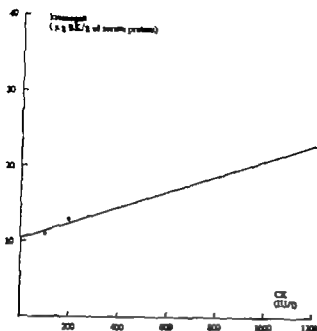


Fig 11 Relation between maximal plasma kininogen decrease (expressed in proportion to serum total protein) and maximal serum total CK activity in acute myocardial infarction ($y = 10.48 + 0.011x$ $r = 0.4365$ $t = 2.829$ $p < 0.01$ $N = 36$)

DISCUSSION

It is generally agreed that certain serum enzyme levels after myocardial infarction roughly correlate with infarct size (Nydick et al 1955 see Sobel et al 1972). In this study maximal serum total creatine kinase was compared with the changes in plasma prekallikrein and kininogen.

The mean appearance time of the peak value of serum total CK activity in the group was 23.7 hours, which is in good agreement with two other reports (Mathey et al 1974, Inoue et al 1977) 24 hours both. The mean maximal total CK value at this time 369 IU/l is also in agreement with the results of other authors (Mathey et al 1974) 380 IU/l.

Because intramuscular injections can cause a release of extracardiac CK (Melzer et al 1970, Zener and Harrison 1974, Klein et al 1973) all patients who received intramuscular injections were excluded from CK-grouping. For the same reason patients who had been resuscitated or defibrillated were also excluded (Konttinen et al 1969). The most severe hemodynamic group including 7 patients was also excluded since extracardiac CK release has also been reported in shock (Klein et al 1973).

It can be seen that the activity is highest in the group with mild to moderate hemodynamic disturbances (Group II) and lowest in the most severe group. When the value of first 24 hours is compared with other values within each group there is a statistically significant decrease in the inhibitory activity on the third to fourth day in Group I ($t=1.742$ df 43 $p<0.05$) and in Group II on the third to fourth day ($t=2.184$ df 33 $p<0.05$). Within Group III the decrease is never statistically significant.

When the three groups are compared with each other it is found that Group I differs significantly from Group II on the second day ($t=1.724$ df 48 $p<0.05$) and after a month ($t=1.810$ df 38 $p<0.05$) and from Group III after a week ($t=1.762$ df 28 $p<0.05$).

In Group II the inhibitory activity is significantly higher than in Group III on the second day ($t=2.187$ df 33 $p<0.05$) and a week after the onset of the disease ($t=2.256$ df 34 $p<0.05$).

Fig. 12 shows plasma prekallikrein in hemodynamic subgroups I-III.

Group I differs from Group II significantly on the third to fourth day ($t=1.751$ df 54 $p<0.05$) and from Group III on the second day ($t=2.074$ df 30 $p<0.05$), the third to fourth day ($t=3.327$ df 30 $p<0.01$) and after a week ($t=2.410$ df 28 $p<0.05$). Group II differs significantly from Group III on the third to fourth day ($t=1.982$ df 36 $p<0.05$).

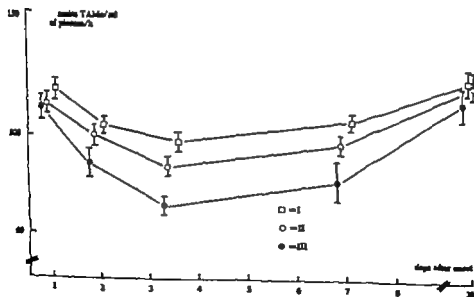


Fig. 12 Plasma prekallikrein (mean and S.E.M.) in acute myocardial infarction grouped according to clinical hemodynamic severity. Group III represents the most severe cases as to

DISCUSSION

According to these results changes in the plasma kinin system are greater in transmural than in non-transmural infarctions. The results of Dzizinski and Kuimov (1972) Kedra et al (1973) and Kolber Postepska (1975) did not show this difference. It is possible that the greater activation is due to a greater mass of myocardial necrosis.

Coronary thrombosis is rare in patients with subendocardial infarction (Roberts and Buja 1972) whereas occlusive thrombosis is more usual in patients with transmural infarction from 54 % (Roberts and Buja 1972) to 95 % (Davies et al 1976).

Changes both in plasma prekallikrein and in kallikrein inhibitory activity are a sensitive indicator of activation of the intrinsic coagulation pathway (Hason and Colman 1971). It is possible that the observed greater changes in transmural infarctions indicate the activation of intrinsic coagulation and subsequent thrombin formation.

IV PLASMA KALLIKREIN INHIBITORY ACTIVITY PREKALLIKREIN AND KININOGEN AS RELATED TO CLINICAL HEMODYNAMIC SEVERITY OF AMI

The patients were divided into three subgroups according to clinical hemodynamic criteria. Group I had no clinical signs of heart failure the criteria of which are stated in chapter Methods (I 2). There were 25 patients in this group. Group II consisted of 33 patients with mild to moderate signs of heart failure and Group III consisted of 7 patients with signs of severe hemodynamic disturbances either of left ventricular failure and/or shock.

Kallikrein inhibitory activity in the hemodynamic subgroups is shown in Table V.

Table V Kallikrein inhibitory activity (IU) in acute myocardial infarction grouped according to clinical hemodynamic severity (means and S.E.M.) Group III represents the most severe cases. See text.

Group (N)	<24h	24-48h	48-96h	6-8d	30d
I (25)	1.10 ± 0.051	1.03 ± 0.037	1.00 ± 0.025	1.02 ± 0.028	1.07 ± 0.033
II (33)	1.19 ± 0.033	1.11 ± 0.032	1.07 ± 0.044	1.07 ± 0.033	1.16 ± 0.038
III (7)	1.05 ± 0.087	.94 ± 0.095	.96 ± 0.040	.89 ± 0.102	.96 ± 0.067

It can be seen that the activity is highest in the group with mild to moderate hemodynamic disturbances (Group II) and lowest in the most severe group. When the value of first 24 hours is compared with other values within each group there is a statistically significant decrease in the inhibitory activity on the third to fourth day in Group I ($t=1.742$ df 43 $p<0.05$) and in Group II on the third to fourth day ($t=2.184$ df 58 $p<0.05$). Within Group III the decrease is never statistically significant.

When the three groups are compared with each other it is found that Group I differs significantly from Group II on the second day ($t=1.724$ df 48, $p<0.05$) and after a month ($t=1.810$ df 38 $p<0.05$) and from Group III after a week ($t=1.762$ df 55 $p<0.05$).

In Group II the inhibitory activity is significantly higher than in Group III on the second day ($t=2.187$ df 33 $p<0.05$) and a week after the onset of the disease ($t=2.256$ df 34 $p<0.05$).

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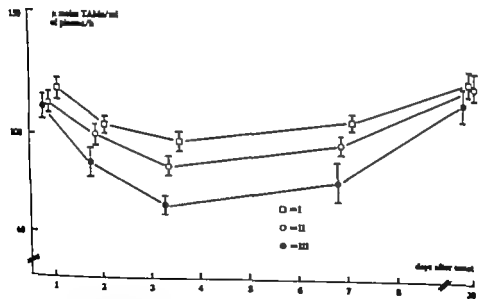


Fig. 12 Plasma prekallikrein (mean and S.E.M.) in 100 myocardial infarcted patients grouped according to clinical hemodynamic severity. Group III represents the most severe cases. S.E.M. = standard error of the mean.

DISCUSSION

According to these results changes in the plasma kinin system are greater in transmural than in non-transmural infarctions. The results of Dzizinski and Kulmov (1972) Kedra et al (1973) and Kolber Postepska (1975) did not show this difference. It is possible that the greater activation is due to a greater mass of myocardial necrosis.

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Changes both in plasma prekallikrein and in kallikrein inhibitory activity are a sensitive indicator of activation of the intrinsic coagulation pathway (Hason and Colman 1971). It is possible that the observed greater changes in transmural infarctions indicate the activation of intrinsic coagulation and subsequent thrombin formation.

IV PLASMA KALLIKREIN INHIBITORY ACTIVITY PREKALLIKREIN AND KININOGEN AS RELATED TO CLINICAL HEMODYNAMIC SEVERITY OF AMI

The patients were divided into three subgroups according to clinical hemodynamic criteria. Group I had no clinical signs of heart failure, the criteria of which are stated in chapter Methods (I 2). There were 25 patients in this group. Group II consisted of 33 patients with mild to moderate signs of heart failure, and Group III consisted of 7 patients with signs of severe hemodynamic disturbances, either of left ventricular failure and/or shock.

Kallikrein inhibitory activity in the hemodynamic subgroups is shown in Table V.

Table V Kallikrein inhibitory activity (IU) in acute myocardial infarction grouped according to clinical hemodynamic severity (means and S.E.M.) Group III represents the most severe cases. See text.

Group (N)	<24h	24-48h	48-96h	6-8d	30d
I (25)	1.10 ± 0.051	1.03 ± 0.037	1.00 ± 0.025	1.02 ± 0.028	1.07 ± 0.033
II (33)	1.19 ± 0.033	1.11 ± 0.032	1.07 ± 0.044	1.07 ± 0.033	1.16 ± 0.038
III (7)	1.05 ± 0.087	0.88 ± 0.095	0.96 ± 0.040	0.89 ± 0.102	0.96 ± 0.067

It can be seen that the activity is highest in the group with mild to moderate hemodynamic disturbances (Group II) and lowest in the most severe group. When the value of first 24 hours is compared with other values within each group there is a statistically significant decrease in the inhibitory activity on the third to fourth day in Group I ($t=1.742$ $df=43$ $p<0.05$) and in Group II on the third to fourth day ($t=2.184$ $df=58$ $p<0.05$). Within Group III the decrease is never statistically significant.

When the three groups are compared with each other it is found that Group I differs significantly from Group II on the second day ($t=1.724$ $df=48$ $p<0.05$) and after a month ($t=1.810$ $df=38$ $p<0.05$) and from Group III after a week ($t=1.762$ $df=28$ $p<0.05$).

In Group II the inhibitory activity is significantly higher than in Group III on the second day ($t=2.187$ $df=58$ $p<0.05$) and a week after the onset of the disease ($t=2.256$ $df=34$ $p<0.05$).

Fig. 12 shows plasma prekallikrein in hemodynamic subgroups I-III.

Group I differs from Group II significantly on the third to fourth day ($t=1.751$ $df=54$ $p<0.05$) and from Group III on the second day ($t=2.074$ $df=30$ $p<0.05$), the third to fourth day ($t=3.327$ $df=30$ $p<0.01$) and after a week ($t=2.410$ $df=28$ $p<0.05$). Group II differs significantly from Group III on the third to fourth day ($t=1.982$ $df=36$ $p<0.05$).

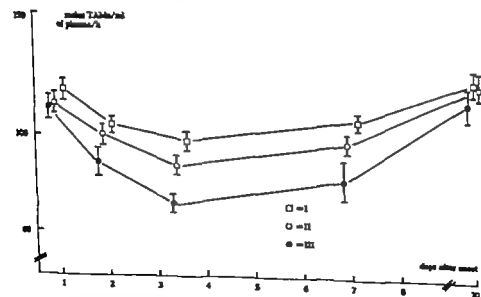


Fig. 12 Plasma prekallikrein (mean and S.E.M.) in acute myocardial infarction grouped according to clinical hemodynamic severity. Group III represents the most severe cases. See text.

As seen in Fig 13 and Fig 14 kininogen drops faster than prekallikrein and reaches the control level sooner. When kininogen is expressed as μg of bradykinin/ml of plasma Group I does not differ significantly from Group II, but with Group III the difference is statistically significant on the second day ($t=2.003$ df 29 $p<0.05$) and the third to fourth day ($t=2.046$ df 30 $p<0.05$). Group II does not differ significantly from Group III.

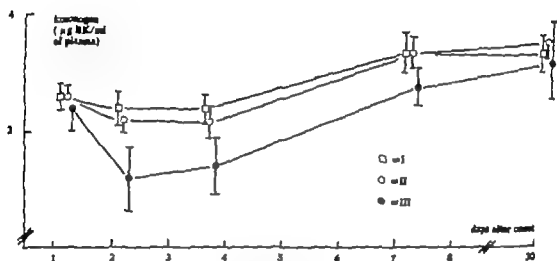


Fig 13 Plasma kininogen (expressed as μg BK/ml of plasma) in acute myocardial infarction grouped according to clinical hemodynamic severity (means and S.E.M.) Group III represents the most severe cases. See text.

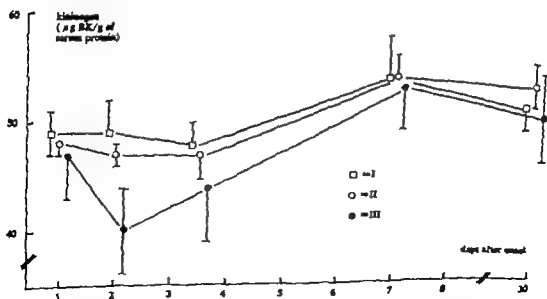


Fig 14 Plasma kininogen expressed as μg BK/g of serum protein) in acute myocardial infarction grouped according to clinical hemodynamic severity (means and S.E.M.) Group III represents the most severe cases. See text.

When corrected in proportion to serum protein (μg bradykinin/g of serum protein) there is however a significant difference between Groups II and III on the second day (t 1.376 df 37 $p < 0.05$)

The time of appearance of maximal kininogen level decrease in Group II (mean 39.2 h) is significantly shorter than in Group III (mean 52.3 h) (t 1.738 df 37 $p < 0.05$)

DISCUSSION

Direct hemodynamic measurements were performed only on those patients in this study who were admitted to the intensive care unit. However with well documented clinical findings clinical classification by an expert cardiologist leads to the correct conclusion in 80 % of the patients as compared with classification made by direct hemodynamic measurements only (Forrester et al 1977)

Thus the subdivision of the patients was performed by a cardiologist according to clinical records which had been completed bedside by the author in each case. 82 % of the patients had at least mild hemodynamic disturbances

Kallikrein inhibitory activity in all subgroups is higher than in the control patients (see Table III) on the first day of the disease with a decline thereafter. Kallikrein inhibition is highest in Group II and lowest in Group III. It is possible that in the most severe cases this compensatory mechanism collapses. In less severe cases it is probably acting normally and most effectively in those patients (Group II) in whom all compensatory mechanisms are expected to be active.

The present findings also confirm that both plasma prekallikrein and kininogen consumption is correlated with the hemodynamic severity of acute myocardial infarction. The later appearance of maximal kininogen decrease in more severe cases suggests a prolonged activation of the plasma kinin system in these cases.

Observations on the association of the hemodynamic severity of infarction with plasma prekallikrein decrease (Gomazkov 1974; Golikov et al 1977) and with plasma kininogen decrease (Sicuteri et al 1967; Ullizinski and Kulmova 1972; Kadane et al 1973; Kolber-Postepska 1975) have been made earlier. Wiegandhausen et al (1967) however noticed no correlation between hemodynamic severity and plasma kininogen consumption.

As seen in Fig 13 and Fig 14 kininogen drops faster than prekallikrein and reaches the control level sooner. When kininogen is expressed as μg of bradykinin/ml of plasma Group I does not differ significantly from Group II but with Group III the difference is statistically significant on the second day ($t=2.003$ df 29 $p<0.05$) and the third to fourth day ($t=2.046$ df 30 $p<0.05$). Group II does not differ significantly from Group III.

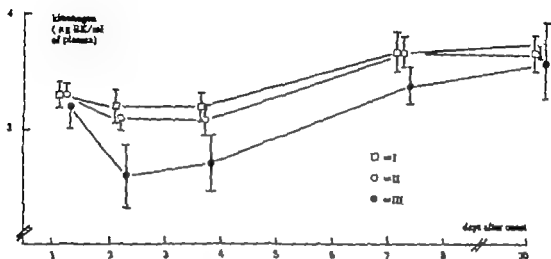


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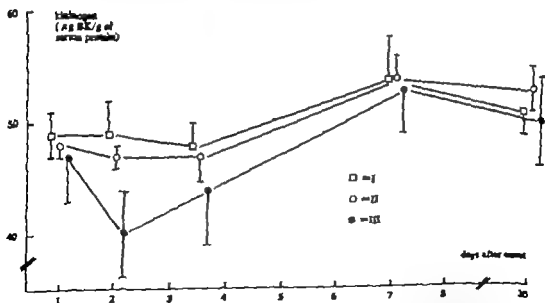


Fig 14 Plasma kininogen expressed as μg BK/g of serum protein) in acute myocardial infarction grouped according to clinical hemodynamic severity (means and S.E.M.) Group III represents the most severe cases. See text.

elevated kallikrein inhibitory activity on the first days after the initial symptoms dyspnea at rest

Pitt et al (1969) assayed the plasma kallikrein system in coronary sinus blood during pacing test in 17 patients. Of 17 patients with signs of myocardial ischemia 7 showed activation of the plasma kallikrein system. Four patients with pacing-induced angina failed to show activation of the plasma kallikrein system and one of the seven who showed activation of the kallikrein system during myocardial ischemia had only ECG changes but no anginal pain.

This along with the present findings suggests that the plasma kallikrein-kinin system alone can hardly be responsible for the pain in myocardial ischemia or infarction.

There are no other reports in the literature on the plasma kinin system and on the presence or duration of pain in clinical myocardial ischemia or infarction.

Kimura et al (1975) examined the relationship between direct hemodynamic parameters and kininogen and kinin levels in infarction patients. They found significant correlations between the lowering of blood pressure, total peripheral resistance and increase of circulation time on one hand, and plasma kininogen decrease and kinin increase on the other.

In the infarction group of the present study there were four patients who died during the stay in hospital. Two died of cardiac arrest and two - after being unconscious for a period of time - of brain edema. Since the group was small and not uniform, no attempt was made to draw any conclusions concerning the changes in the plasma kinin system.

V PLASMA PREKALLIKREIN AND KININOGEN AS RELATED TO THE DURATION OF PAIN IN ACUTE MYOCARDIAL INFARCTION

The onset of pain was carefully recorded by asking each patient personally and checked from the patient records. The duration of pain was estimated using the need of analgesics, mostly morphine, as an indicator.

No attempt was made to evaluate the degree of pain owing to its subjective nature.

A direct comparison between each patient's duration of pain and the maximal prekallikrein and kininogen decrease or their time of appearance did not show any correlation.

DISCUSSION

The mean duration of pain in the group was 23.8 hours, which agrees well with reports by other authors. Inoue et al (1977) obtained by asking each patient's complaints of chest pain a mean of 24 hours for the duration of pain in acute myocardial infarction.

The findings of this study are not sufficient to show that activation of the plasma kinin system correlates with the presence and/or duration of pain in myocardial infarction.

There was only one patient who experienced no pain. He had electrocardiographic changes of extensive antero-lateral infarction, maximal serum total CK activity of 793 IU/l, and severe hemodynamic disturbances. This patient showed marked activation of the plasma kinin system: a decrease in both prekallikrein and kininogen, as well as

elevated kallikrein inhibitory activity on the first days after the initial symptoms of dyspnea at rest

Pitt et al (1969) assayed the plasma kallikrein system in coronary sinus blood during pacing test in 17 patients. Of 11 patients with signs of myocardial ischemia 7 showed activation of the plasma kallikrein system. Four patients with pacing induced angina failed to show activation of the plasma kallikrein system and one of the seven who showed activation of the kallikrein system during myocardial ischemia had only ECG changes but no anginal pain.

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There are no other reports in the literature on the plasma kinin system and on the presence or duration of pain in clinical myocardial ischemia or infarction.

GENERAL DISCUSSION AND CONCLUSIONS

65 patients with acute myocardial infarction admitted to Helsinki University Central Hospital were included in the study. Patients over 75 years of age, alcoholics, and patients who died within two days after admission were excluded. Four patients included in the study died during the stay in hospital. In other respects the patients represented a typical unselected sample of acute myocardial infarction.

16 hospitalized control patients in no way connected with the infarction group were included in order to evaluate the effect of hospitalization and immobilization which appeared not to influence the plasma kallikrein-kinin system.

The activation of the plasma kinin system should lead to a decrease in plasma prekallikrein and kininogen with a concomitant rise in plasma kallikrein and bradykinin. Simultaneous changes in both plasma kallikrein inhibitory activity and kininase activity are also to be expected. Plasma free bradykinin was not measured in this study. All the other components of the plasma kinin system mentioned above were determined during the course of acute myocardial infarction, i.e. first, second and third or fourth day, one week and one month from the onset of the acute symptoms.

It was concluded that the plasma kinin system is activated during the acute stage of myocardial infarction. This conclusion was based on the observation that both plasma prekallikrein and kininogen decrease. Moreover, in the very early stage of the disease kallikrein inhibitory activity increases.

No changes in spontaneous TAME-esterase (kallikrein) activity were observed in this study. It is, however, possible that there is an increase in free kallikrein but it either takes place very early during the disease and is thus missed, or it is suppressed by the increased kallikrein inhibitory activity. It is also possible that changes are not

observed owing to the relative insensitivity of the method. The observed decreases in plasma prekallikrein and kininogen agree with earlier observations by others. In previous studies no attention has been paid to possible hemodilution or hemoconcentration due to disturbances in the patients fluid balance. This possibility along with the observation that serum total protein decreased during the disease led to the expression of plasma kininogen values also in proportion to serum total protein. The changes remained significant. This is taken as a proof that the decrease is actual and further that it is probably due to kininogen consumption and not to any nonspecific decrease caused by impaired protein synthesis.

Changes in kininase activity during myocardial infarction were found. The method used measures only the spontaneously occurring kininase activity in plasma. It is possible that the marked kininase potential of the lungs for example may easily overcome the kinin splitting activity needed and thus no changes in plasma kininase activity need to occur.

In addition to clear signs of the activation of the plasma kinin system in acute myocardial infarction it was found that the magnitude of the changes in plasma prekallikrein and kininogen were in correlation with the severity of the infarction. Consequently it was observed that the prekallikrein and kininogen decrease is greater when maximal serum total CK activity is higher and also when the infarction is transmural as compared with the non-transmural infarctions.

This suggests that the changes in the plasma kinin system are related to the size of the infarction.

The changes in plasma prekallikrein and kininogen were also greater when the clinical hemodynamic situation was more severe. Moreover the appearance of maximal kininogen decrease takes place significantly later in more severe cases which indicates longer duration of the activation of the plasma kinin system.

Kallikrein inhibitory activity was high on the first day of the disease possibly reflecting an early change in the plasma kinin system. In transmural infarctions inhibitory activity was significantly higher than in non-transmural infarctions. In hemodynamic subgroup II with mild to moderate hemodynamic disturbances kallikrein inhibitory activity was higher than in the other subgroups.

One reason for the interest in the role of the plasma kinin system in ischemic heart disease has been the possibility that the plasma kinins could be the direct cause of ischemic pain.

In this study no correlation was found between either the duration or the degree of the activation of the plasma kinin system on one hand and the duration of pain on the other.

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SUMMARY

This study was carried out in order to investigate the plasma kinin system during the course of acute myocardial infarction. 65 infarction patients hospitalized through the outpatient department of Helsinki University Central Hospital were included. Patients over 75 years of age, alcoholics and patients who died within two days after admission were excluded. Blood samples from each patient were collected on admission, on the second and third or fourth days, and after one week and one month from the onset of the acute symptoms. The following blood analyses were carried out: plasma spontaneous esterase (TAME) activity (kallikrein), plasma prekallikrein, plasma kallikrein inhibitory activity, plasma kininogen and plasma kininase activity, as well as serum total creatine kinase (CK) activity during the first three days after admission. Serum CK MB-isoenzyme measurement was carried out in order to make a final differentiation in doubtful cases of myocardial infarction. Serum total protein was determined from all samples. A control group of 16 hospitalized patients undergoing a minor eye operation was included to evaluate the influence of hospitalization and immobilization. The samples were taken on admission, on the second day, and on the third or fourth day in hospital. There were no changes in the examined components of the plasma kinin system in this group. In the infarction group spontaneous esterase activity (kallikrein) remained unchanged. Plasma prekallikrein decreased from the first day on, being at its lowest on the third to fourth day of the disease, and still significantly below the initial and control levels after one week. Plasma kallikrein inhibitory activity was significantly elevated on the first day of the disease, as compared with the control values. Plasma kininogen decreased, being at its lowest on the third to fourth day, but returned to the control level in one week. These differences remained significant even after the correction of the kininogen values in proportion to the serum total protein, which also showed a significant

Thus the role of the plasma kinin system as a cause of ischemic pain was not confirmed by the present results. It is however possible and indeed likely - that the pain producing action of the kinins in ischemia is a local phenomenon and cannot be detected by measurements of circulating components of the kinin system.

SUMMARY

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reduction in the course of the disease. Plasma kininase activity did not change during the disease and did not differ from that of the control group. The severity of the infarction was evaluated by maximal serum total CK activity and by clinical hemodynamic criteria as well as by dividing the patients into transmural and non transmural infarction groups according to ECG criteria. There was a significant correlation between the plasma prekallikrein and kininogen decrease and the maximal serum total CK activity. The patients were divided into three subgroups according to clinical hemodynamic criteria. In the first group there were no signs of hemodynamic disturbances. In the second group there were mild to moderate signs of hemodynamic disturbances and in the third group the disturbances were severe. It was found that the decrease in plasma prekallikrein and kininogen is greatest in the third group and smallest in the first group. Moreover, the time of appearance of the maximal decrease in plasma kininogen took place significantly later in the third group than in the second group. Plasma kallikrein inhibitory activity was highest in the second group and lowest in the third group. Plasma prekallikrein and kininogen decrease was significantly greater and plasma kallikrein inhibitory activity significantly higher in the group with transmural infarction than in the group with non transmural infarction. No correlation was found between the duration of pain and the magnitude and the time of appearance of the maximal decrease in plasma prekallikrein and kininogen.

On the basis of this study it is concluded that the plasma kinin system is activated in the course of acute myocardial infarction and that the magnitude of the changes in the components of the plasma kinin system is correlated with the extent and clinical severity of the infarction but not with the presence or duration of pain.

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